EXERCISE-INDUCED ORGAN PROTECTION IN A MURINE MODEL OF CARDIAC ARREST AND RESUSCITATION

BY

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ABSTRACT

Cardiac arrest causes whole-body ischemic injury and cell death. Successful cardiopulmonary resuscitation paradoxically confounds recovery by increasing the rate of cellular death and tissue damage through global reperfusion injury. Despite decades of basic and clinical research, the prognosis after a resuscitated cardiac arrest continues to be poor. The substantial effects of cardiac arrest on neurologic function are a major contributor to the high incidence of mortality following resuscitation.

Engaging in moderate-intensity aerobic exercise 24 to 72 hours prior to prolonged ischemic exposure can create a prophylactic, conditioned response that minimizes tissue damage. This exercise-induced effect resembles protection conferred by surgical induction of a series of brief, sub-lethal ischemic episodes, known as ischemic preconditioning. The unpredictability of cardiac arrest renders ischemic preconditioning impractical and useless as a neuroprotective defense mechanism. In light of this impracticality, aerobic exercise could provide the only reasonable preventative measure for inducing sustainable organ protection from ischemic injury.

Furthermore, characterization of the murine electrocardiogram during and immediately following resuscitated cardiac arrest is exceedingly limited in the current literature. Thus, a clear knowledge gap exists in the methodology of murine models of arrest which we attempted to fill.

We hypothesized that exercise preconditioning can confer neuroprotection against prolonged, global ischemia associated with cardiac arrest. Additionally, we proposed that real-time electrocardiographic pattern recognition in the first 30 seconds post-arrest

can be used to predict survival in a murine model of cardiac arrest and resuscitation. We tested these hypotheses using male C57Bl/6J mice 10-12 weeks of age in a potassium-induced model of arrest. The mice were trained in a forced treadmill exercise training protocol pre-arrest and neurologic function was serially tested. In the post-arrest period, neurologic testing was repeated to detect changes in cognitive function and emotionality.

Our results showed that real-time ECG pattern recognition is a reliable tool for determining the success of resuscitation efforts. Key characteristics of survival emerged in the visual appearance of the RR interval, PR interval, QRS complex, heart rate, and the relation of the J-point to the isoelectric line. These characteristics were substantiated on *post-hoc* quantitative analysis. Furthermore, as predicted, exercise preconditioning confers neuroprotection during cardiac arrest. This was evidenced by a lower fraction of hippocampal neuronal apoptosis compared with non-exercised animals and a concomitant preservation of retrograde memory.

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LIST OF ABBREVIATIONS

AMI	Acute myocardial infarction
AP	Action potential
CA	Cardiac arrest
CA_1	Cornu Ammonis 1
Ca^{2+}	Calcium
CON	Control group
COX-2	Cyclooxygenase-2
CPR	Cardiopulmonary resuscitation
CVD	Cardiovascular disease
ECG	Electrocardiogram
EPI	Epinephrine
Ex+CA	Exercise preconditioning + cardiac arrest
ExPC	Exercise preconditioning
HR	Heart rate
I/R	Ischemia-reperfusion
iNOS	Inducible nitric oxide synthase
IPC	Ischemic preconditioning
K^+	Potassium
KCl	Potassium chloride
MnSOD	Manganese superoxide dismutase
Na^+	Sodium
OFA	Open Field Activity
ROSC	Return of spontaneous circulation
ShEx+CA	Sham exercise + cardiac arrest
SR	Sarcoplasmic reticulum
VF	Ventricular fibrillation
VO_{2max}	Maximal oxygen consumption

CHAPTER 1

INTRODUCTION

1.1 Cardiac Arrest Statistics: Incidence, Risk and Survival

Cardiac arrest (CA) causes whole-body ischemic injury and cell death. Successful cardiopulmonary resuscitation (CPR) paradoxically confounds recovery by increasing tissue damage and the rate of cellular death through global reperfusion injury. Despite decades of basic and clinical research, the prognosis after a resuscitated arrest continues to be poor.

Emergency Medical Services-treated, out-of-hospital CA occurs in approximately 235,000 to 325,000 Americans annually.[1] Underlying cardiovascular etiology is responsible for 60 to 70 percent of the arrests,[2] with the strongest risk factors being hypertension, known coronary artery disease, type 2 diabetes mellitus, family history of coronary heart disease, a history of smoking, and poor cardiorespiratory fitness.[3]

Rates of survival following CA are dismal. Although 20 to 50 percent of out-of-hospital CA patients have achieved the return of spontaneous circulation (ROSC) at hospital admission, only a mere 6 to 10 percent of CA patients survive to hospital discharge.[1, 4-6] The rate of survival is highly dependent on geographical locale and patient characteristics, however, and ranges from 0.08 percent (Delaware County, IN) to 25 percent (Rochester, MN).[4]

1.3. Post-Cardiac Arrest Syndrome: Overview and Pathophysiology

Survivors of CA often develop a multi-system dysfunction termed Post-Cardiac Arrest Syndrome.[7-9] This is a complex pathophysiological process that includes post-arrest brain and myocardial damage in combination with systemic ischemia-reperfusion injury and a persistent underlying pathology.[2, 7] Interestingly, the substantial effects of CA on

neurologic function carry much of the burden of mortality. A retrospective study of CA patients indicated that neurologic injury was responsible for death in 68 percent of out-of-hospital arrests and 23 percent of in-hospital arrests.[10] By comparison, only 23 percent of out-of-hospital CA patients died of cardiovascular causes, though cardiac etiology tends to be the major precipitating factor.[10]

1.3.1 Neurologic Sequelae of Cardiac Arrest

The pathophysiology of neurologic dysfunction is initiated during the arresting period and can persist for days following successful resuscitation.[7, 8, 11] The total cessation of cerebral blood flow at the onset of arrest results in ATP depletion, reactive oxygen species formation and cytokine release. These factors initiate injury cascades that lead to cellular membrane destruction, electrolyte derangement and excitotoxic injury.[7, 8, 11] Acidosis development occurs rapidly and the blood-brain barrier begins to breakdown under the stress of reactive oxygen species accumulation and lipid peroxidation. Neuronal damage in concert with the release of apoptogenic factors leads to subsequent cell death.

Blood flow is partially restored at the onset of reperfusion, however cardiac output is still significantly reduced resulting in impaired cerebral blood flow.[8] The increase in platelet activation and blood coagulation that occurs during the arrest period is augmented during reperfusion leading to microthrombi production and accumulation in the cerebral microvessels. Endothelial dysfunction coupled with thrombus formation leads to altered perfusion and contributes to the no-reflow phenomenon prolonging the ischemic period.[7, 8, 11]

Impaired cerebrovascular autoregulation in the immediate post-resuscitation phase allows for hyperemic reperfusion secondary to elevated cerebral perfusion pressures.[7] Hyperemic reperfusion exacerbates the cellular damage attributed to reactive oxygen species that was initiated at the onset of CA. This cellular damage leads to further cerebral edema and apoptosis, thus setting the stage for persistent neuronal damage and increasing neurologic dysfunction.

1.3.2. Myocardial Stunning

Post-resuscitation myocardial stunning is the mechanical cardiac dysfunction that persists after ROSC.[9] Myocardial stunning is characterized by systolic and diastolic dysfunction in the presence of normal coronary blood flow which typically resolves within 24 to 48 hours after onset.[7, 12-15]

Similar to the pathogenesis of neurologic damage, myocardial stunning begins immediately with the onset of arrest. The injury cascades involved begin with an increase in circulating catecholamines, decreased oxygen delivery, increased cytokine and complement activation, and decreased lymph flow.[9] From these catalysts follows a sequence of cellular degradation, electrolyte derangement and reactive oxygen species generation that leads to increased myocardial stiffness, mitochondrial swelling, impaired contractility and increased myocardial edema.[9] The two major contributors to post-CA stunning involve oxyradical formation and the disruption in calcium homeostasis.[14, 15]

During resuscitation, reactive oxygen species are generated by the injured myocytes and increase the membrane damage that was initiated during arrest. The excitation-contraction coupling sequence of cardiac muscle follows the calcium-induced calcium

release cascade, thus cellular membrane damage that exacerbates the calcium overload leads to the formation of a reperfusion contracture.[9, 15] The contracture further increases the myocardial stiffness with concomitant decreased compliance. Similar to cerebral reperfusion complications, the no-reflow phenomenon is also observed in the myocardium and is generally attributed to coronary spasms and increased circulating catecholamine levels leading to pronounced vasoconstriction. Administration of exogenous epinephrine during CPR efforts can further contribute to the potent vasoconstriction.

Treatment for post-arrest myocardial stunning is frequently confounded by the underlying cardiovascular disease common in arrest patients. Because stunning results in systolic and diastolic impairments that mimic the effects of an acute myocardial infarction (AMI), distinction between the transient injury and the permanent dysfunction can be difficult.

1.4. Persistent Neurologic Functional Impairment

Long term deficits in neurologic function plague the majority of individuals that survive the immediate recovery period and reach hospital discharge.[16, 17] A study of patients one year after CA revealed significant, persistent difficulties in memory, behavior and perception.[18] A growing body of research indicates the lasting cognitive impairments associated with CA are linked to hippocampal damage.[19-23] Specifically, the Cornu Ammonis 1 (CA_1) region of the hippocampus appears to be the area most susceptible to severe ischemic damage.[19, 24-26]

1.4.1. The Hippocampus: A mastermind of cognition and emotionality

In 1988, Barnes et al[27] analyzed the role of the hippocampus in reference and working memory as it pertains to spatial learning and cue learning. **Reference memory** is the ability to remember information or cues from one trial to the next. For instance, a mouse will remember to search for an escape hole placed next to a red sign on the wall because that is where the escape hole was located on a previous trial. Thus, associating the successful completion of a task with a visual cue will cause the hippocampus to create a cognitive map of success based on that experience. In this example, a decrease in the latency to escape correlates with an increase in reference memory. Also relevant to this example is the location of the red sign. If the sign were part of the maze, this would be an example of *cued learning*. However, with the red sign as part of the environment external to the maze itself, this becomes an example of *spatial learning*. **Working Memory**, in contrast to reference memory, is the ability to remember information within trials. For instance, a mouse will remember unsuccessfully attempting to escape at the yellow sign rather than the red sign and refrains from repeating this error. Decreasing the number of repeated errors during each trial demonstrates an increase in working memory.[27] These are key variables in examining the total picture of memory and learning and are captured through the use of the Barnes Maze.

Barnes et al[27] was able to assess that damage to the hippocampus appears to be essential for spatial learning but not for cue learning.[27] The researchers noted that when the hippocampus was damaged before or after training on a spatial learning task, deficits were found in both working and reference memory. By contrast, when the

hippocampus was damaged before or after training on a cue learning task, neither working nor reference memory was affected.[27] Twenty-two years after Barnes published these landmark findings on the relationship between the hippocampus, memory, and learning, Travis et al[23] went further to identify the presence of retrograde versus anterograde memory deficits. These researchers were able to demonstrate that cognitive maps generated before CA are subject to memory loss, however those created after the ischemic event tend to remain intact.[23, 28]

Taken together, the findings of Barnes et al[27] and Travis et al[23] have important clinical implications. The discovery of the relationship between hippocampal development and cognition allows for the association of cognitive dysfunction with hippocampal damage. Because the hippocampus is one of the selectively vulnerable areas for ischemic injury, it follows that the most commonly reported post-CA neurologic deficit is persistent retrograde memory impairment.[17]

1.5. The Dual Role of Exercise: Prevention and Preconditioning

1.5.1. The Physiology of Exercise in Cardiovascular Disease

Exercise has been studied extensively as a therapeutic measure for lowering the risk of mortality due to cardiovascular disease (CVD) and in preventing the development of the traditional major CVD risk factors.[29, 30] Notably, exercise is capable of positively affecting every modifiable risk factor except smoking; however, exercisers tend to not be chronic smokers.[31] The pathogenesis involved in hypertension, hypercholesterolemia and hyperlipidemia, diabetes, and obesity, can all be slowed, reversed or prevented by regular exercise.[29] Importantly, cardiorespiratory fitness is an

independent predictor of CVD-related mortality.[3, 30] This is not surprising given the emerging data indicating that the cardioprotective benefits of exercise are disproportionate to the relationship between exercise and risk factor modification.[29, 30] Research by Mora et al[32] indicates that only 60 percent of the decrease in CVD can be attributed to exercise-induced reductions in the major CVD risk factors. This leaves 40 percent of exercise-induced cardioprotection unaccounted for by risk modification. Emerging science regarding this remaining 40 percent has focused on endothelial and autonomic nervous system function.[33, 34] The vascular endothelium is a key site for cardiovascular control as it relates to vasoactivity and subsequent control of perfusion pressures and systemic vascular pressures.[30] DeSouza et al[33] demonstrated a significant reduction in endothelial function associated with aging in a population of young and old sedentary individuals. However, when aged-exercisers were compared with young exercisers, there was no difference in endothelial function.[33] This very vividly speaks to the benefit of exercise on endothelial function.

Furthermore, altered autonomic function has a strong impact on cardiovascular disease pathogenesis.[34] Increased sympathetic nerve activity is apparent in patients with cardiovascular disease and presents as an increase in systemic pressure.[35] Interestingly, in spite of the potent vasoconstricting effects of increased sympathetic outflow, chronic exercisers have been shown to have high sympathetic activity and yet they remain normotensive.[35] This seeming contradiction is likely due to a decrease in α-adrenergic sensitivity and increased endothelial function.[36] In individuals with high levels of sympathetic output, Charkoudian et al[37] demonstrated a significantly higher response in mean arterial pressure when endothelial function was blocked by a nitric

oxide inhibitor. This finding reveals that, when considered in isolation, increased sympathetic activity is not damaging, however, when in conjunction with poor endothelial function this alteration induces hypertension and increases the risk of CVD development.[30, 37]

The influence of exercise on the pathogenesis and prevention of CVD and its associated risk factors is of great importance when considering exercise-induced protection during CA. The pathophysiology of arrest, though considerably more acute, in many ways mirrors the development of CVD. Primarily, the arrest phase is marked by significant sympathoexcitation and endothelial dysfunction leading to pronounced vasoconstriction in the post-arrest phase and prolonging the period of ischemic damage **(FIGURE 1)**. Because the underlying cause of arrest is primarily of cardiac etiology, it is probable that endothelial and sympathetic dysfunction is present prior to the arresting event, and that CA simply exacerbates these issues. It begs the question, then, can an acute bout of exercise protect against the precipitating endothelial and autonomic dysfunction as well as the acutely exacerbated disturbances in order to provide protection during CA?

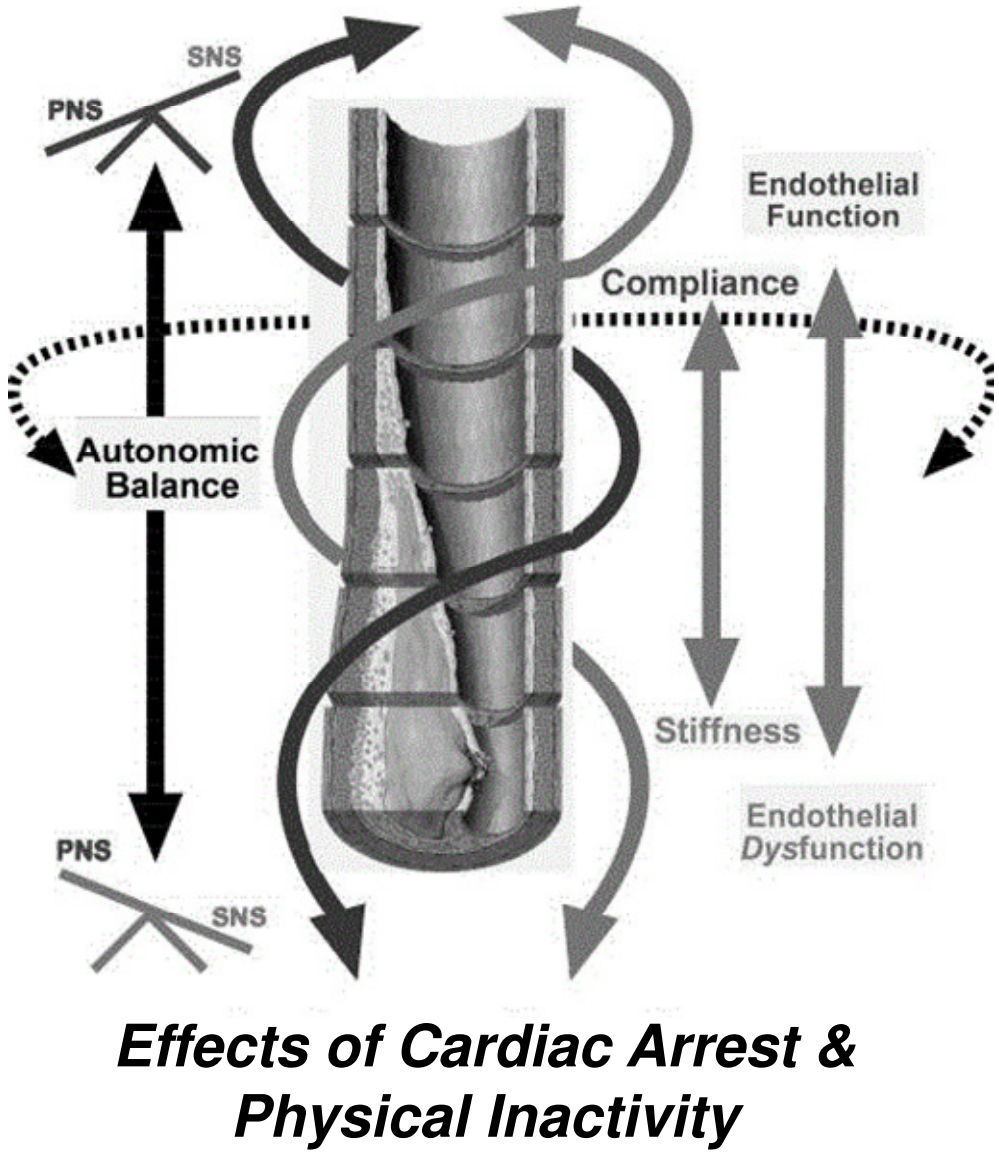

FIGURE 1. Endothelial and autonomic function during exercise and cardiac arrest. In response to exercise, endothelial function and parasympathetic tone are enhanced. Large conducting vessels remain compliant, and the effects of high sympathetic outflow, when present, are buffered. These positive interactions may account for observations showing that exercise is more protective against cardiovascular risk than predicted by its effects on traditional risk factors. As a result of cardiac arrest or in response to physical inactivity, there is a loss of endothelial function and increased vessel stiffness. These effects permit the high sympathetic tone to be more fully expressed while parasympathetic tone is progressively lost. Reprinted and modified with permission, Joyner and Green.[30]

1.5.2. Preconditioning

Physiologic preconditioning refers to the exposure of an organ or organ systems to a sub-lethal stress in order to generate a protective phenotype against a subsequent, lethal stress. Preconditioning occurs in two phases: early and late. Early preconditioning is initiated within minutes of the removal of the original stressor and lasts approximately 3 h.[38, 39] Late preconditioning, is activated approximately 12 to 24 h after the initial stress and lasts approximately 48 to 72 h. Several mechanisms of preconditioning have been described in the literature including ischemic, pharmacologic and exercise-induced.[31, 38, 40-42]

1.5.3. Comparing Preconditioning Methods: Practicality, Sustainability, Durability

Ischemic preconditioning (IPC) refers to exposure to a brief, sub-lethal ischemic episode which renders the body resistant to a future ischemic event.[38] This preconditioning method is commonly employed in surgical scenarios where disruption of blood flow is required such as during coronary artery bypass grafting. The molecular basis of IPC has been attributed to an upregulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).[43-45] This discovery of the mechanistic pathways leading to IPC has been used in the development of pharmacologic preconditioning methods via the use of nitrates and adenosine A1 receptor agonists, specifically.[39, 43, 45, 46] Finally, exercise preconditioning (ExPC) mirrors IPC in time to onset and extent of protection, but differs mechanistically and in duration.[47-49]

Quindry et al[48] confirmed that both acute and chronic exercise training confers cardioprotection during transient, focal ischemia in acute myocardial infarction (AMI),

however COX-2 is not upregulated in young or senescent animals in response to exercise training. The same study found no relation between iNOS expression and exercise.[48] The lack of increase in these defense-induced proteins in response to exercise suggests that the body perceives exercise as a manageable stressor, and ischemia as a potentially lethal, or distressing stressor.[48] These findings allow exercise to be labeled as a "stress without distress" stimulus, which induces positive physiologic adaptations. In light of the lack of relation between exercise and iNOS or COX-2, other molecular pathways have been explored to identify the underpinnings of ExPC. Most prominently, heat shock proteins and antioxidants have received extensive attention for their anti-apoptotic and anti-inflammatory potential.[40, 50-55]

The duration of effectiveness is also a point of difference between IPC and ExPC. In studies of cardioprotection during AMI, the window of protection afforded by IPC extends approximately 72 h.[38, 56, 57] In contrast, the potency of ExPC has extended its effectiveness to at least 9 d following the last exercise bout.[58] Furthermore, all potential mediators of ExPC, including heat shock proteins and the antioxidant manganese superoxide dismutase (MnSOD), were within normal limits while cardioprotection was still activated.[58] Similar studies on the duration of IPC or ExPC and neuroprotection have not been conducted.

Lastly, the mechanistic disparity between ExPC and IPC may explain why they differ in the extent of effectiveness across the lifespan.[48, 59, 60] Shulman et al[60] demonstrated a significant loss of IPC effectiveness in a middle-aged population consistent with decrements in the corresponding signaling cascade involving iNOS and COX-2. Through the prevention of calpain and caspace 3 activation, ExPC has experimentally

been shown to decrease myocardial apoptosis in young and old hearts.[59] These findings have significant implications on the development of pharmacomimetics for inducing preconditioning effects in aged individuals.[60]

While useful in surgical scenarios, the unpredictability of CA renders IPC impractical and useless as a defense mechanism in out-of-hospital CA. For this reason, exercise provides the only reasonable preventative measure for inducing sustainable organ protection during CA. Importantly, the above findings are based on models of transient, focal ischemia consistent with AMI; confirming the effectiveness of ExPC during severe, prolonged global ischemia inherent in CA was a major goal of this research project.

1.5.4. Exercise Preconditioning: Acute response or chronic adaptation

Studies on the effects of exercise on cardio- and neuroprotection have varied significantly in the intensity and duration of exercise protocols employed.[42, 52, 61, 62] Intensity of exercise relates to the required oxygen consumption to maintain a specific workload. To that extent, intensities ranging between 55 and 75 percent maximum oxygen consumption (VO_{2max}) have been used to determine the effects on cardioprotection in studies of ischemia and reperfusion (I/R).[63, 64] Interestingly, Lennon et al[31, 64] found no difference in the level of protection afforded by moderate (55 percent VO_{2max}) and high (75 percent VO_{2max}) intensity exercise. However, Starnes et al[63] was unable to demonstrate cardioprotection at intensities of 55-60 percent VO_{2max}. The devil in the details of these two studies is hidden in the length of each exercise bout. The study by Lennon et al[64] required sustained exercise for 60 min per session compared with 40 min in the study by Starnes et al.[63] Given this distinction, it would appear that exercise duration *and* intensity are important for eliciting organ protection.

Furthermore, the overall study period has been investigated to determine the period of exercise necessary to elicit cardio- and neuroprotective phenotypes.[40, 59, 61, 62, 65, 66] Notably, studies of neuroprotection have indicated a training period of three weeks or greater to consistently elicit a protective phenotype;[40] however exercise intervals of two weeks show less consistent results,[61, 62] and periods of one week or less have not produced any significant results.[62] Interestingly, results for studies of cardioprotection are not consistent with those of neuroprotection. Cardioprotection has been demonstrated using exercise protocols ranging from three days to 16 weeks.[65-67]

The above protocols were used to determine the adaptation of a protective phenotype in models of I/R; however no studies have determined the effectiveness of short-term exercise on eliciting such protection in models of CA. Furthermore, it should be noted that, while the above studies are presuming to address ExPC, the length of the exercise protocols would suggest chronic adaptations are being evaluated, rather than acute responses. By nature, ExPC is a short duration event occurring within 24 h of a previous stress. By this definition, extensive exercise protocols spanning weeks would likely be cardio- and neuroprotective due to physiologic adaptations such as angiogenesis, neurogenesis, enhanced functional sympatholysis, and increases in mitochondrial antioxidant capacity and heat shock protein expression. In studies of persistent exercise, it is difficult to discern the effects produced by the most recent bout of exercise and those generated by the cumulative effects of an exercise training regimen. To this end, our lab sought to determine the effectiveness of two bouts of exercise within 48 h of the arresting event. A drawback, however, was the need to acclimate the animals to the treadmill which occurred over a five day period with two

days of rest between acclimation and testing. Drawing the exercise period out over 7 days however, still would not allow for chronic adaptations in blood flow mechanics or neurologic functioning through angiogenesis or neurogenesis.

1.6. Characterizing a Murine Model of Cardiac Arrest

The study of CA requires the development of an animal model that mimics the true physiologic conditions of arrest, resuscitation and recovery. Large animal models more easily employ coronary occlusion prior to the arresting stimulus giving the results greater translational value and clinical relevance.[68] Murine models, on the other hand, allow for transgenic manipulation and capitalization on potential biomarkers for the development of therapeutic measures.

1.6.1. Generating the Electrocardiogram: The Importance of electrolyte balance

The standard electrocardiogram (ECG) instantaneously provides a window into the electrical function of cardiomyocytes and is wholly dependent on a constant and predictable flux in sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}). The propagation of an action potential (AP) begins with rapid cellular depolarization due to the opening of voltage-gated, fast Na^+ channels. The open Na^+ channels allow for an influx of Na^+ along the electrochemical gradient. This influx represents phase 0 of the AP. Within milliseconds of opening, the Na^+ channels close and voltage-gated, slow Ca^{2+} channels are opened in the sarcolemma and the sarcoplasmic reticulum (SR). This increases the permeability of Ca^{2+} allowing its concentrations to increase in the cytosol. The movement of Ca^{2+} and the small counterbalancing outflow of K^+ create the plateau phase (phase 2) of the AP. After a delay, voltage-gated K^+ channels open increasing

the membrane permeability to K^+. Simultaneously, the Ca^{2+} channels close leaving K^+ to reset the resting membrane potential (AP phases 3 and 4).

Excitation-contraction coupling translates the AP into the production of tension (e.g., contraction). As Ca^{2+} enters the cell during the plateau phase, it signals the release of Ca^{2+} from the SR (i.e., Ca^{2+}-induced Ca^{2+} release). This SR-released Ca^{2+} binds to Troponin C on the actin myofilament revealing myosin binding sites resulting in cross-bridge attachment and contraction.

Translation of the electrochemical AP into the electromechanical action of tension generation is captured by the electrocardiogram (ECG) and results in distinct waves associated with depolarization and repolarization. The first ECG deflection reflects atrial depolarization and is represented as the P wave. The P wave is followed by a biphasic waveform associated with ventricular depolarization referred to as the QRS complex. This wave generates considerably greater amplitude than the P wave due to the difference in tissue mass between the atria and the ventricles. The QRS complex is followed by a relatively isoelectric segment, the ST-segment, reflecting the time lapse between ventricular depolarization and repolarization. Ventricular repolarization is reflected as the low-amplitude T wave.

Alterations in electrolyte balance disturb the propagation and rate of the AP, and this translates into changes in the duration of corresponding segments on the ECG. Likewise, necrotic tissue does not conduct electrical impulses, so instances of tissue damage alter the direction of the waveforms and, when using a 12-lead ECG system, allow for the identification of the infarcted region.

1.6.2. Finding Repolarization: The Anomaly of the Murine Electrocardiogram

The increased development and use of murine models of acute cardiac events underscores the need for a more complete understanding of associated ECG changes. Importantly, natural variations between human and murine ECGs make visual characterization of abnormalities challenging.[9, 69, 70] The major differentiating point in waveform depiction occurs during ventricular repolarization.[71-73] The distinct T wave and largely isoelectric ST-segment of humans gives way to a nearly non-existent ST-segment followed by a high amplitude secondary QRS notch in mice **(FIGURE 2)**. This notch represents the fast portion of ventricular repolarization and thus would correlate with the human T wave.[70, 72, 73] The distinct attachment point of the T wave to the QRS represents the J-point and is the key point of inference when determining ST-segment elevation or depression in a murine ECG.[71]

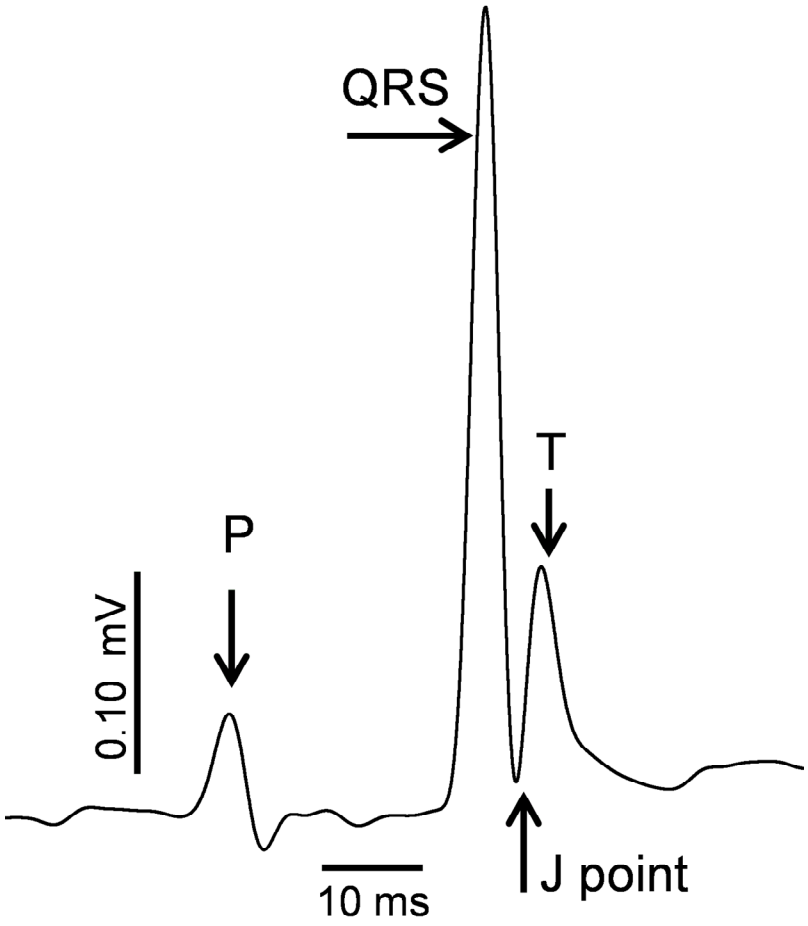

FIGURE 2. Electrocardiographic representation of a single murine heartbeat. Notice the lack of an isoelectric ST-segment which creates a prominent J-point. The mouse T wave is indicative of the initial stage of ventricular repolarization.

1.6.3. Characterizing the Insult: Hypoxia, Ischemia and Hyperkalemia on the ECG

The ECG is the most immediate tool for the assessment of resuscitation efforts and the prediction of post-CA survival. By nature CA is a hypoxic-ischemic event capable of producing large infarcted regions or severely stunned myocardium, depending on the duration of the cardiac event. Cardiac arrest causes cellular membrane degradation and electrolyte derangement. These disturbances are identified by characteristic changes in the ECG. Additionally, the experimental induction of CA by potassium chloride (KCl) injection could compound the distinction of the physiologic responses to arrest by altering patterns of depolarization and repolarization.

Hypoxia: Hypoxia, in the absence of ischemia, produces a marked decrease in heart rate, and significant prolongation of the PR interval and QT interval.[74, 75] If left untreated, the PR interval changes increase in severity to the point of complete heart block.[74] These findings are associated with prolonged repolarization consistent with alterations in the balance of Ca^{2+} and K^{+} and elongation of phase 2 of the AP.[75, 76]

Ischemic Hypoxia: The compound effects of hypoxia and ischemia are experimentally modeled through ligation of the left coronary artery which induces a focal ischemia capable of mimicking the effects of AMI. This experimental condition produces progressive increases in J-point elevation and a pronounced prolongation of the QT interval.[69, 77] Elevation of the ST-segment (or J-point in the murine ECG) is indicative of sarcolemmal K^{+} channel dysfunction. These changes appear within the first few minutes of ischemia and continue throughout the ligation period. Prolonged ischemia associated with permanent coronary occlusion or the induction of CA causes T-wave inversion coupled with QRS prolongation.[78] The change in T wave appearance is

indicative of an infarcted region which alters the path of electrical conduction and changes the direction of the electrical deflection. Prolongation of the QRS complex is due to an electrolyte derangement causing a decrease in excitability.[78]

Reperfusion following short-duration (e.g. 30 min) focal ischemia or 12 min of global ischemia recovers the pre-ischemic waveform patterns.[77, 78] However, prolonged focal ischemia (e.g. >1 hour) produced ECG changes that were not reversed by reperfusion.[77] Further prolongation of the ischemic event led to irreversible T wave inversion, pathologic Q waves and voltage abnormalities consistent with permanent coronary occlusion and severe infarction.[77] These dominant features of the permanently occluded coronary artery are indicative of changes in the polarity of depolarization due to impediments from infarcted cardiomyocytes. Additionally, necrotic cells tend to release their inner contents thus disrupting the electrolyte balance of the surrounding environment and effecting AP propagation.

Hyperkalemia: Experimental models of CA employing KCl as the arresting agent potentially confound ECG interpretation through non-arrest-related changes in depolarization and repolarization expressed as electromechanical dysfunction. Normal reference limits for potassium in C57Bl/6J mice ranges between 3.4 and 5.5 mEq/L.[79] Evidence of hyperkalemia in models of KCl-induced CA have reported K^+ levels twice that of the reference range.[80] The relevance of hyperkalemia in ECG interpretation relates to the significant changes in T wave appearance, QRS duration, and J-point elevation,[81] which must be deciphered from those changes directly related to the hypoxic-ischemic insult and reperfusion injury. Because K^+ is directly responsible for repolarizing the myocytes and maintaining the resting membrane potential, significant

increases in extracellular K^+ will interfere with repolarization and significantly elevate the resting membrane potential.[81] The increase in resting membrane potential alters the number of Na^+ channels available for activation which slows the AP and prolongs the QRS complex.[81] Adequate CPR pushes residual K^+ through the system to be filtered and released, as a result, the ECG can be used to determine the effectiveness of resuscitation efforts.

1.7. Research Questions

In light of the evidence supporting the preventative and medicinal value of exercise and the substantial use of mice in models of acute cardiac events, the two-fold purpose of our study was to characterize ECG changes associated with survival in a murine model of CA and to determine if ExPC could extend its neuroprotective benefits to include global ischemic events. The following specific aims were used to test the research questions:

Specific Aim 1: To determine if real-time ECG characterization during the initial 30 s of ROSC could be used to determine the effectiveness of CPR and to predict survival. *We hypothesized that specific and distinct ECG patterns associated with post-CA survival exist and that these patterns are evident regardless of time-to-ROSC. Further, we hypothesized that the real-time analysis would be substantiated by post-hoc quantitative analysis.*

Specific Aim 2: To determine the effectiveness of short-term, moderate-intensity exercise in conferring neuroprotection during CA. *We hypothesized that two consecutive days of exercise training at 70 percent of VO_{2max} would elicit an*

endogenous neuroprotective response to CA confirmed on functional neurologic assessments and hippocampal apoptosis evaluations.

CHAPTER 2

ELECTROCARDIOGRAPHIC CHARACTERIZATION OF SURVIVAL IN A MURINE MODEL OF POTASSIUM-INDUCED CARDIAC ARREST AND RESUSCITATION

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Manuscript in Preparation

2.1 Abstract

Experimental modeling of severe cardiovascular conditions is an integral component of developing clinically relevant therapeutic measures. For such purposes, murine models of advanced cardiovascular diseases and acute cardiac events such as cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) have been developed. The extensive cellular damage, electrolyte derangement and area of infarct in the CA and CPR model limits successful return of spontaneous circulation (ROSC) and survival. Real-time pattern recognition of the standard electrocardiogram (ECG) provides the most immediate tool for the assessment of resuscitation efforts and the prognostication of post-CA survival. The purpose of this study was to determine the reliability of real-time ECG pattern recognition in the initial 30 s of ROSC in predicting survival outcomes. Secondarily, we suggest *post-hoc* quantitative waveform analysis could substantiate the visual assessments. Cardiac arrest was induced in 28 male C57Bl/6J mice via infusion of potassium-chloride (KCl; 80 µl, 0.5M). After maintaining 5 min of asystole, CPR was conducted using mechanical ventilation, chest compressions and injection of epinephrine. Needle-probe ECG analogous to Lead III was continuously recorded and monitored for pattern recognition. Our results show that significant and consistent ECG patterns associated with survival occur within 30 s of ROSC, and often within the initial 10 s of ROSC. These changes were quantified on *post-hoc* analysis and were defined by alterations in RR interval, heart rate, QRS duration, and J-point between survivors and non-survivors. The mouse model of CA can be improved by using real-time ECG pattern recognition to determine the effectiveness of resuscitation efforts.

2.2. Introduction

Survival subsequent to cardiac arrest (CA) is dependent on adequate perfusion stimulated through cardiopulmonary resuscitation (CPR) and the physiologic response to such efforts. Importantly, the return of spontaneous circulation (ROSC) does not guarantee survival or a continued sustainable rhythm. The standard electrocardiogram (ECG) instantaneously provides a window into the impact of resuscitation attempts. This diagnostic tool has the potential to serve as the most immediately available real-time predictor of post-CA survival.

Unfortunately, few reports exist regarding the visual characterization of ECGs in murine models of CA and resuscitation despite the wide use of mice in describing the physiologic implications of prolonged, global ischemia.[9, 69, 74, 77, 78, 82] The natural variation of the murine ECG relative to humans has been consistently reported in the literature with a major point of emphasis delineating ventricular depolarization from repolarization.[70-73] Briefly, the ECGs of large mammals express ventricular repolarization with a distinct T wave following a largely isoelectric ST-segment. In contrast, the T-wave in mice represents only the initial, fast phase of ventricular repolarization[70, 72, 73] and is seemingly attached to the QRS complex (e.g. ventricular depolarization), in the absence of an isoelectric ST-segment, by the J-point (**FIGURE 2**).[71] Murine ECG changes have been used to confirm experimental models of focal I/R,[77] hyperkalemia[81, 82] and hypoxia,[74] but not, to our knowledge, to characterize survival in models of CA and CPR.

Real-time ECG pattern recognition could provide key physiologic feedback to guide the resuscitation efforts and differentiate between survival and non-survival rhythms. We

hypothesized that the first 30 s of ECG characteristics could accurately predict the achievement of sustainable ROSC indicative of post-resuscitation survival in the absence of quantitative data. Secondarily, we used *post-hoc* analysis to quantify the ECG features of survivors and non-survivors in an attempt to further classify ECG responses to CA and resuscitation efforts.

2.3. Materials and Methods

Experimental protocols were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

Twenty-eight male, C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) 10-12 wks of age, weighing 24.75 ± 0.49 g were anesthetized with 60 mg kg^{-1} sodium pentobarbital to surgical depth. The mice were orally intubated with a 20-gauge catheter, the placement of the tracheal tube was verified, and mechanical ventilation was initiated (MiniVent; Hugo Sacks, March, Germany). Body temperature was maintained by a heating pad and monitored by a rectal thermocouple probe (Physitemp Instruments, Inc., Clifton, NJ). A 27-gauge needle was inserted into the right jugular vein for administration of potassium-chloride (KCl) and epinephrine (EPI). Needle-probe ECG analogous to Lead III was recorded and continuously monitored with the use of a PC-based data acquisition system (PowerLab Inc., ADInstruments, Sydney, Australia) for the duration of arrest, resuscitation and 15 min of recovery. Beyond 15 min, 30 s ECG recordings were obtained every 5 min until 30 min post-arrest.

To induce CA, room temperature KCl (80 µl, 0.5 M) was infused and ventilation was discontinued. At 4 min 45 s into the arrest period, ventilation was resumed. At 5 min of

arrest, CPR was initiated via chest compressions (approximately 300 beats per min, bpm) with administration of 5 µg of EPI in 0.25 ml saline. ROSC was defined as an unassisted pulse at a rate greater than 200 bpm that was maintained, unassisted, for at least 1 min (**FIGURE 3)**. The jugular catheter was removed at 10 min post-ROSC and the wound sutured. After 90 min of recovery, mechanical ventilation was discontinued, and the mice were extubated and placed in a recovery cage until sedation subsided.

Initial distinction of survivors versus non-survivors was made using visual cues within the ECG waveforms during the first 30 s of ROSC. Regardless of ECG appearances, however, survival techniques (e.g. chest compressions, EPI administration) were applied as necessary for a period of 10 min following CA. If an animal was unable to sustain an unassisted rhythm by the end of 10 min, all survival attempts were discontinued. Cardiopulmonary resuscitation was resumed and additional EPI administered, up to 0.25 additional ml, for any animal that achieved ROSC but subsequently lost the ability to sustain a perfusing rhythm within the initial 10 min post-arrest period. Resuscitation efforts were not continued beyond 10 min regardless of rhythm.

Continuous ECG recordings were sampled at 4kHz s^{-1} and analyzed using 4-beat averages. Because the ST-segment lies within the junction of the QRS complex and T waves, references to ST-segment elevation reflect J-point elevation. The RR interval, HR, PR interval, QRS complex duration, Q-wave amplitude (Q_{Amp}), ST-segment elevation (ST_{Elev}) and QT variables were used for *post-hoc* quantification of ECG waveforms and overall rhythm analysis at three distinct time-points: 0-30 s (T1), 30-60 s (T2), 60-90 s (T3) of ROSC. A 200 Hz low-pass filter was applied during all ECG

acquisitions to reduce artifact and baseline drift. Two consistent sources of artifact apparent throughout the recordings were the electricity of the heating pad located beneath the animal, and the rhythmic movements derived from mechanical ventilation. During CPR an additional source of artifact stemmed from the movement of the upper extremity electrode with each chest compression. To minimize this, the upper extremity electrode was taped in place. All artifact was corrected for during *post-hoc* analysis and had no effect on waveform quantification.

The primary outcome of interest was the ability to accurately identify survivors and non-survivors based on visual cues offered by the ECG in the initial 30 s of ROSC. Secondary outcomes included the overall quantification of ECG waveforms and the subsequent comparison of survivors and non-survivors. Repeated measures ANOVA was used to compare waveform values at specific time points. The ECG data of survivors and non-survivors were compared using one-way ANOVA for T1, T2, and T3. Differences were assessed by means of the unpaired Student t-test between each group at every stage. Quantitative analysis was performed with the use of LabChart 7 (PowerLab Inc, ADInstruments, Sydney, Australia) and transferred to Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA) for assessment of statistical significance.

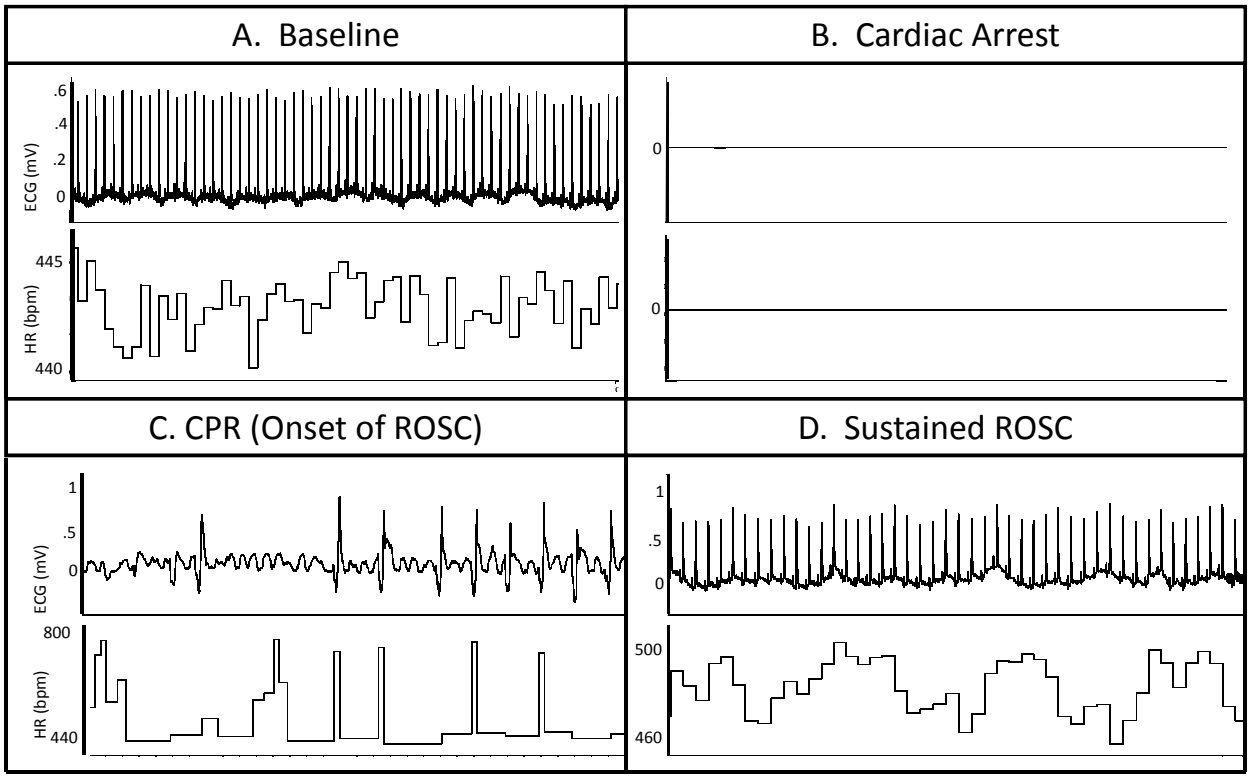

FIGURE 3. Electrocardiographic sequence from arrest through resuscitation**.** Representative electrocardiographic and heart rate recordings prior to arrest (A), during the arresting period (B), at the transition from CPR to a self-sustainable rhythm (C), and after 5 min of sustained ROSC.

2.4. Results

All animals achieved ROSC for the defined minimum of 1 min, however not all animals were supported by a sustainable rhythm. Thus, a dichotomy was created between animals able to maintain a perfusing rhythm unassisted (survivors, n=17) and those who could not (non-survivors, n=11); the data were analyzed using this natural division of survival. At baseline, the groups did not significantly differ in weight (p=0.113), age (p=0.773), core temperature (p=0.861), or on any ECG variable (RR interval, p=0.998; heart rate [HR], p=0.863; PR interval, p=0.0945; QRS complex duration, p=0.240; Q_{Amp}, p=0.998; ST_{Elev}, p=0.294; QT, p=0.758) ***(TABLE 1).*** Total arrest time was not significantly different between groups (p=0.383) and therefore did not contribute a confounding factor for survival.

Between-group differences existed in ECG patterns and were identifiable in real-time within 30 s of ROSC. Waveforms corresponding with survival exhibited (i) distinct P, QRS, and T waves, (ii) biphasic waveforms of low amplitude ranging between -0.5 and 0.5 mV morphing into predominantly positive deflections between 0.5 and 1.0 mV, (iii) a narrowing of the QRS complex within 10 s of the onset of ROSC, and a decrease in RR interval duration and variability relative to non-survivors **(FIGURE 3, A-G).** Non-survivors presented with (i) absent P waves and ST segments, (ii) QRS with a predominantly negative deflection and amplitude ranging between -1.15 and 0.5 mV, (iii) unresolved RR interval variability throughout the first 30 s that progressively dissolved into 2° and 3° atrioventricular blocks requiring further resuscitation efforts, (iv) wide-complex ventricular rhythms with accompanying bradycardia, (v) sinoventricular pacing collapsing into ventricular fibrillation (VF) and asystole **(FIGURE 4, A-G).**

Post-hoc analyses detailed in ***TABLES 2 and 3*** quantitatively confirm the real-time evaluation. Between-group differences in RR interval, HR, PR interval, and QRS complex duration were significant at each 30 s interval in the initial 90 s of ROSC **(TABLE 2).** In survivors, the RR interval was significantly decreased at each period (T1 and T3, $p<0.005$; T2, $p<0.01$) and HR significantly increased (T1and T2, $p<0.001$; T3, $p<0.005$) when compared with non-survivors. Likewise, the QRS complex duration (T1 and T3, $p<0.005$; T2, $p<0.01$) and QT interval (T1 and T2 $p<0.05$; T3, NS) were significantly shortened in survivors versus non-survivors. **TABLE 3** outlines within-group differences and indicates conflicting changes in RR interval, HR, PR interval, and QRS complex duration relative to pre-arrest values between survivors and non-survivors. Thus, survivors experienced a shortening of the RR interval (T1, T2, and T3 $p<0.001$) and QRS complex duration (T1 and T2, $p<0.001$; T3, $p<0.05$) with a corresponding increase in HR (T1, $p<0.05$; T2 and T3, $p<0.001$). In contrast, non-survivors presented with an increasing RR interval (T1, $p<0.05$; T2, NS; T3, $p<0.01$), a progressively increased QRS complex duration (T1, T2, and T3, $p<0.001$) and a low, relatively stable HR (T1 and T2, NS; T3, $p<0.05$) that eventually led to electromechanical dissociation.

TABLE 1. Baseline physiological characteristics by survival outcome. No between-group differences existed at baseline. Survivors, n=17; non-survivors, n=11.

	Age (wks)	BW (g)	Temp (°C)	Arrest Time	RR (ms)	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	ST (mV)
Non-Survivors	10.91	23.78	36.80	8:00	160.48	382.19	35.87	10.63	20.41	31.36
±SEM	0.25	0.61	0.06	0:27	6.63	16.45	1.11	0.38	0.82	11.08
Survivors	11.00	25.37	37.20	7:28	160.51	386.36	33.98	11.16	19.86	44.87
±SEM	0.19	0.67	0.02	0:17	6.78	17.19	0.51	0.25	0.96	7.19

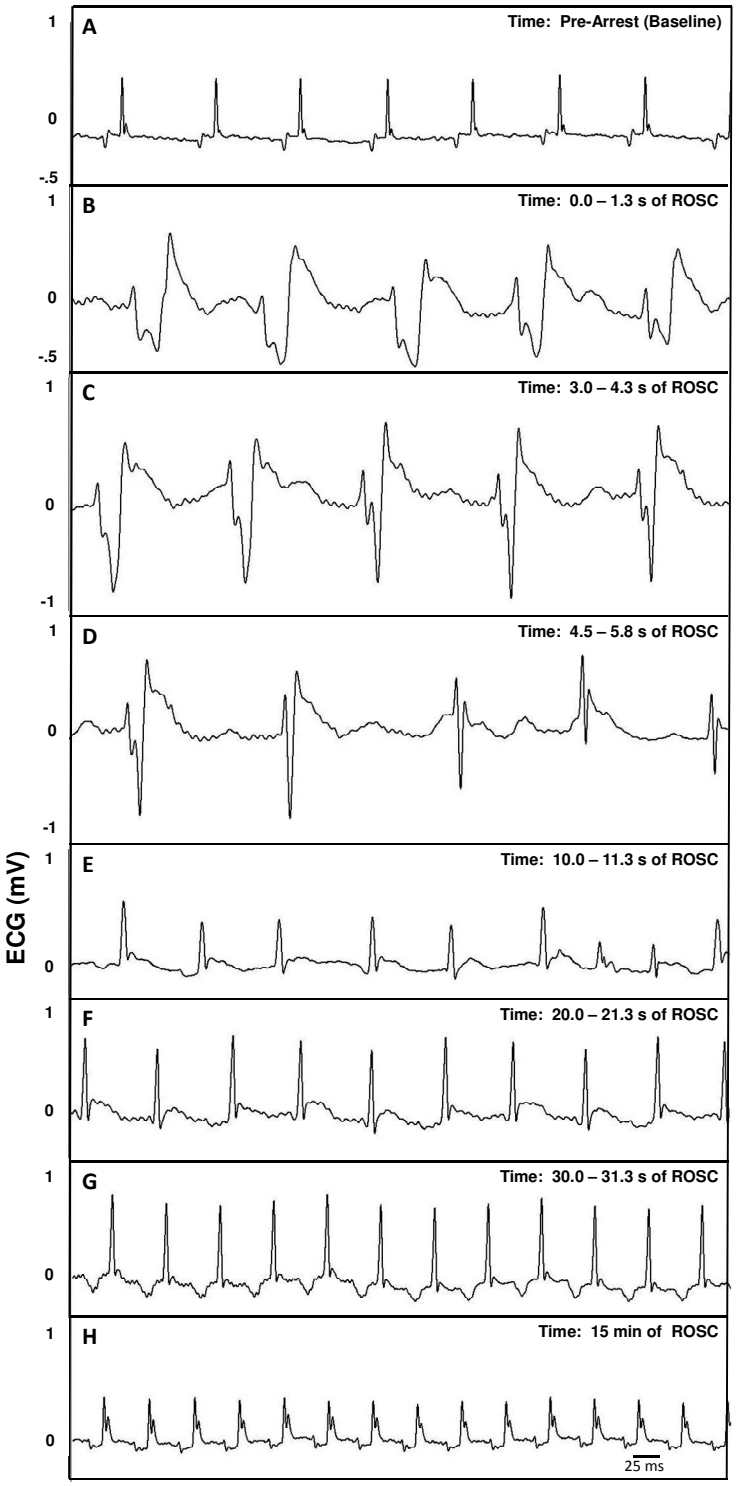

FIGURE 4. Representative ECG of a single survivor depicting the initial onset of ROSC through the first 6 s (*A*-*D*), and at 10 s (E), 20 s (F), 30 s (G), and 15 min (H) of recovery.

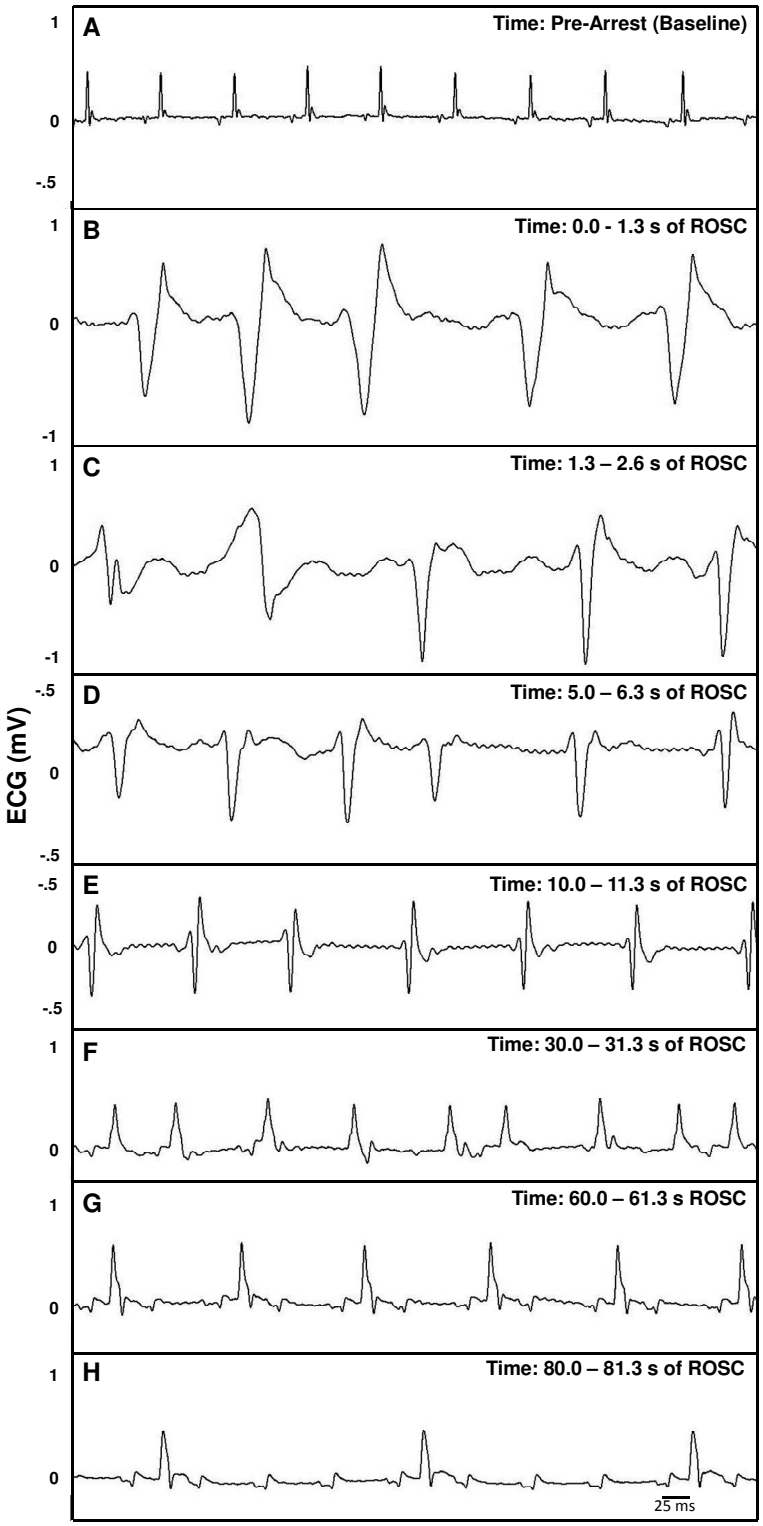

FIGURE 5. Representative ECG of a single non-survivor depicting the initial onset of ROSC through the first 6 s (*A*-*D*), and at 10 s (*E*), 30 s (*F*), 60 s (*G*) and 80 s (*H*) of recovery.

TABLE 2. Between-group differences in ECG variables during the initial 90 s post-arrest. [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.005$; [d] $p<0.001$ compared to non-survivors at the same time-point. Numbers in parenthesis represents ±SEM. Survivors, n=17; Non-survivors, n=11

	Baseline		Onset of ROSC (T1)		30 s after ROSC (T2)		60 s after ROSC (T3)	
	Non-Survivor	Survivor	Non-Survivor	Survivor	Non-Survivor	Survivor	Non-Survivor	Survivor
RR (ms)	160.48 (6.63)	160.51 (6.78)	200.34 (10.91)	151.16 (3.91)[c]	195.14 (23.06)	116.41 (2.37)[b]	234.51 (31.39)	111.97 (1.82)[c]
HR (bpm)	382.19 (16.45)	386.36 (17.19)	341.48 (15.95)	427.38 (8.03)[d]	346.18 (31.63)	521.25 (9.95)[d]	326.99 (44.08)	538.21 (8.69)[c]
PR (ms)	35.87 (1.11)	33.98 (0.51)	18.95 (1.47)	23.18 (1.09)[a]	24.36 (3.22)	36.77 (1.45)[b]	29.25 (2.30)	37.23 (0.91)[a]
QRS (ms)	10.63 (0.38)	11.16 (0.25)	30.40 (1.37)	23.64 (1.34)[c]	24.34 (2.43)	15.48 (1.31)[b]	21.64 (1.99)	13.78 (1.00)[c]
QT (ms)	20.41 (0.82)	19.86 (.096)	47.42 (1.72)	42.15 (1.86)[a]	42.09 (4.03)	28.54 (1.95)[a]	42.92 (7.45)	27.78 (2.72)
QTc (ms)	51.96 (2.59)	50.56 (3.17)	106.45 (6.65)	114.25 (6.86)	92.78 (8.95)	83.38 (5.75)	93.07 (11.57)	82.78 (7.52)
Q_{Amp} (mV)	7.73 (2.48)	7.73 (1.90)	-0.275 (.075)	-0.127 (.032)	-0.235 (.115)	1.45 (17.68)	-0.084 (.065)	.0385 (.015)
ST (mV)	31.36 (11.08)	44.87 (7.19)	-0.065 (.062)	0.015 (.021)	0.0212 (.063)	36.86 (18.29)	-0.045 (.075)	0.0622 (.016)

TABLE 3. Within-group differences in ECG variables during the initial 90 s post-arrest. [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.005$; [d] $p<0.001$ compared to baseline. Numbers in parenthesis represents ±SEM. Survivors, n=17; Non-survivors, n=11.

		Baseline	Onset of ROSC (T1)	30 s after ROSC (T2)	60 s after ROSC (T3)
Non-Survivors	RR (ms)	160.48 (6.63)	200.34 (10.91)[a]	195.14 (23.06)	234.51 (31.39)[c]
	HR (bpm)	382.19 (16.45)	341.48 (15.95)	346.18 (31.63)	326.99 (44.08)[a]
	PR (ms)	35.87 (1.11)	18.95 (1.47)[d]	24.36 (3.22)[a]	29.25 (2.30)[b]
	QRS (ms)	10.63 (0.38)	30.40 (1.37)[d]	24.34 (2.43)[d]	21.64 (1.99)[d]
	QT (ms)	20.41 (0.82)	47.42 (1.72)[d]	42.09 (4.03)[c]	42.92 (7.45)
	QTc (ms)	51.96 (2.59)	106.45 (6.65)[a]	92.78 (8.95)[b]	93.07 (11.57)
	Q_{Amp} (mV)	7.73 (2.48)	-0.275 (.075)[c]	-0.235 (.115)	-0.084 (.065)
	ST (mV)	31.36 (11.08)	-0.065 (.062)	0.0212 (.063)	-0.045 (.075)
Survivors	RR (ms)	160.51 (6.78)	151.16 (3.91)[d]	116.41 (2.37)[d]	111.97 (1.82)[d]
	HR (bpm)	386.36 (17.19	427.38 (8.03)[a]	521.25 (9.95)[d]	538.21 (8.69)[d]
	PR (ms)	33.98 (0.51)	23.18 (1.09)[d]	36.77 (1.45)[d]	37.23 (0.91)[b]
	QRS (ms)	11.16 (0.25)	23.64 (1.34)[d]	15.48 (1.31)[d]	13.78 (1.00)[a]
	QT (ms)	19.86 (.096)	42.15 (1.86)[d]	28.54 (1.95)[d]	27.78 (2.72)[a]
	QTc (ms)	50.56 (3.17)	114.25 (6.86)[d]	83.38 (5.75)[d]	82.78 (7.52)[c]
	Q_{Amp} (mV)	7.73 (1.90)	-0.127 (.032)[d]	1.45 (17.68)	.0385 (.015)
	ST (mV)	44.87 (7.19)	0.015 (.021)	36.86 (18.29)	0.0622 (.016)

2.5. Discussion

This investigation has demonstrated clear ECG configurations associated with survival in the initial 30 s of ROSC following KCl-induced CA. The success of ECG pattern recognition was substantiated by *post-hoc* quantitative analysis of waveform arrangement. It is worth noting that while no significant difference existed between groups, the range of time-to-ROSC extended from 1:02 to 5:16 of CPR and yet the rules of ECG pattern recognition were applicable across the range. This is strong evidence of the utility of real-time pattern recognition for determining survivorship in the initial seconds of ROSC.

The three essential distinguishing features differentiating survival from non-survival via real-time ECG pattern analysis were the width of the QRS complex, HR, and ST-segment alterations. The initial wide QRS complex commonly observed in survivors at the onset of ROSC is similar in structure to waveforms associated with permanent occlusion in a murine model of AMI.[77] The distinct appearance of the waveform **(FIGURE 4, A-B)** when analyzed under experimental conditions of AMI has been attributed to poor reperfusion and tends to persist long-term. In the current model of CA, however, the abnormal wave pattern resolves into a sustainable, narrow, biphasic QRS complex within seconds of the onset of ROSC **(FIGURE 4, A-C).** This conversion into a survival pattern is suggestive of adequate perfusion and demonstrates effective CPR.

Heart rate is continuously provided by the data acquisition system, but is also easily distinguishable in real-time. **FIGURE 4G** and **FIGURE 5F** both represent a 1.3 s screen shot at 30 s of ROSC. Without quantification it is clear, based on the number of beats

falling into each image, that the survivor **(FIGURE 4G)** has a higher HR compared to the non-survivor. The HR response by 30 s in survivors is invariably higher than baseline levels and tends to continue increasing throughout the first several minutes post-ROSC. To qualify this finding, the baseline HR tends to be slightly suppressed due to the anesthetic cocktail which could give the recovery HR an artificially elevated appearance. Regardless, within the first 10 s of ROSC a survivors' HR is significantly higher than both baseline and its non-surviving counterpart's rate, and can easily be used as part of the visual prediction of survival.

Ventricular repolarization of survivors typically experiences the greatest conformational change throughout the course of recovery. The ST-segment tends to begin as elevation in conjunction with the abnormal QRS. Once the wide QRS complex narrows, the ST-segment is altered to appear relatively isoelectric. Finally, the segment reverts into a prominent degree of J-point elevation by the end of the initial 15 minute recovery phase. ST-segment elevation, here defined by the J-point, is the definitive hallmark of acute MI.[69, 77] However, in studies of CA without prior coronary ligation, myocardial stunning is considered a more likely culprit for the observed ST-segment elevations.[9] Unlike the elevation in acute MI which has been shown to be related to alterations in potassium and ATP,[69] ST-segment alterations in myocardial stunning are primarily a function of calcium overload,[78] a known transient after-effect of CA.[9]

The ECG of non-survivors appears in direct opposition to that of survivors relative to the key variables of QRS complex duration, HR and ST-segment elevation. As shown in **FIGURE 5, A-G**, the QRS complex of non-survivors is distinctly wider than their surviving counterparts. This widening effect could be due to severe hyperkalemia

generated by the combined effects of ischemic damage and exogenous KCl administration.[9, 80, 81] Although evidence of hyperkalemia has previously been indicated in models KCl-induced CA and might be a methodological limitation,[80] it could also plausibly reflect ineffective reperfusion during CPR and a failure to clear the KCl from the cardiovascular system.[83]

The morphing of the wide and strongly biphasic QRS complex into a predominantly negative QS wave is a trademark distinction of a non-sustainable rhythm. This pattern change tends to be coupled with an absence of P waves and a consumption of the J-point within the QS complex. Taken together, these transformations identify issues indicative of ionic imbalances and contractile dysfunction.[9, 69, 71, 74, 78, 81, 84] Ventricular tachycardia and fibrillation arise out of these electrical and mechanical insufficiencies. Occasionally the predominance of a negative QS gives way to an upright RS wave indicating a change in the direction of ventricular depolarization. In isolation, this would generally signify an improvement in myocardial function, however the QRS complex formation is only one of the three essential variables determining survival outcome, and this shift usually occurs outside the 30-s window of distinction.

The slower HR clearly identifiable in real-time allows for recognition of RR interval variability. **FIGURE 5, D-G** plainly demonstrates the dysrhythmia and intrusion of ectopic foci common to non-survivors. Over the course of the first minute of ROSC, these rhythm disturbances will deteriorate into complete atrioventricular dissociation such that no common pacing mechanism is available **(FIGURE 5, G)**. In real-time, the slowed HR makes distinct beat-to-beat variability easy to visualize, and an initial diagnosis of dysrhythmia simple. In essence, the slow HR coupled with RR interval

irregularity are in direct contrast to the quickness and regularity of the survivor's HR **(see FIGURE 4F**).

Interestingly, the ECG patterns of non-survivors exhibit characteristics similar to those found in cases of myocardial stunning specifically attributable to neurologic dysfunction.[85, 86] Neurogenic myocardial stunning could account for RR interval variability, ST-segment changes, widening of the QRS duration, bradycardia, and an increased likelihood of arrhythmogenesis.[85, 86] Additionally, the brain-heart connection has been extensively critiqued as of late and studies have indicated prominent ECG changes attributable to neurologic dysfunction in the absence of myocardial injury.[85] Thus, it is plausible that the high level of brain damage experienced during the arresting period is responsible for the lack of rhythm sustainability found in non-survivors. Correspondingly, animals with the distinct ECG pattern of a non-survivor at ROSC were unable to be successfully resuscitated regardless of additional efforts made throughout the first 10 min post-arrest. While this could be a negative consequence of EPI overload,[87-89] it may instead indicate critical neurologic deterioration affecting autonomic function and cardiac stability.

A significant strength of the study is that it extends the knowledge of ECG adaptations beyond the realm of short-duration, focal ischemia. Focusing on clinical applicability, however, does illuminate some limitations within the model.

Full and instantaneous arrest of non-cardiac etiology is uncommon in the chronicles of out-of-hospital CA. This is a limiting factor associated with the use of KCl as the arresting agent. Approximately 60 to 70 percent of CA is attributable to advanced

coronary artery disease.[2] The best methodology to adequately mimic the effects of CA, then, would be to first reproduce the prolonged focal ischemia of acute MI and then add the complication of CA. The technical intensity of enacting such a methodology in a murine model of CA exceeds the practicality of the mouse model, however this approach has been successfully utilized in larger animals and has proven to be more representative of human arrest.[68] In the absence of surgical induction of cardiovascular disease, using a murine model prone to common CVD risk factors such as obesity, type 2 diabetes and hypertension, may be able create a non-surgically induced underlying pathology more characteristic of a clinical model.

Secondly, the animals in this study were maintained in normothermia unlike a significant portion of the recent literature which focuses on therapeutic hypothermia, or, at the least, maintains all organs other than the brain in a state of hypothermia to limit global organ damage.[80, 90, 91] Analyzing the physiologic effects of CA on organ function requires an acceptance of the interplay between organ systems which influences whole-body outcomes and is reflected in ECG alterations. The significance of maintaining the mice within the range of normal body temperatures is evident in the discussions of neurogenic myocardial stunning and autonomic uncoupling.

Lastly, four major experimental models of murine CA are used extensively: KCl, electrical induction of ventricular fibrillation, asphyxiation, and exsanguination. Due to the significant hemodynamic differences induced in each model and the differences in the robustness of global ischemia produced, it is unknown whether the ECG survival patterns identified in this study are generalizable to all models of arrest. A comparison of ECG manifestations between the various methods of arrest is warranted.

2.6. Conclusion

When used to its fullest potential, the standard ECG harbors real-time predictive value that provides immediate physiologic feedback useful in determining the effectiveness of resuscitation efforts. Electrocardiographic pattern recognition can be used consistently in the identification of survival from KCl-induced CA. Moreover, *post-hoc* scrutiny of waveform design offers quantifiable data for the real-time descriptors of successful resuscitation and lends credence to the power of ECG pattern recognition.

CHAPTER 3

THE EFFECTS OF EXERCISE PRECONDITIONING ON MEMORY, LEARNING AND ANXIETY IN A MURINE MODEL OF POTASSIUM-INDUCED CARDIAC ARREST AND RESUSCITATION

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Manuscript in Preparation

3.1. Abstract

Cardiac arrest (CA) causes whole-body injury associated with ischemia and reperfusion (I/R). Engaging in moderate-intensity exercise 24 to 72 hours prior to ischemic exposure can create a prophylactic, conditioned response that minimizes tissue damage during transient, focal ischemia. We hypothesized that exercise preconditioning (ExPC) can confer neuroprotection against the prolonged, global ischemia associated with CA. This hypothesis was tested using male C57Bl/6J mice 10-12 weeks of age. Neurologic assessments pre- and post-CA included evaluation of cognition based on retrograde and anterograde spatial learning and memory on the Barnes Maze, and emotionality assessments of anxiety-like behaviors in the Open Field test. Pre-arrest exercise training was conducted using forced treadmill exercise at 15 m/min for 60 min on the two consecutive days prior to CA. Arrest was induced with 80 µl of 0.5 M potassium chloride followed by resuscitation with mechanical ventilation, chest compressions and epinephrine administration. Our results indicate that ExPC confers neuroprotection during CA as evidenced by a lower fraction of hippocampal neuronal apoptosis compared with non-exercised animals, and a subsequent preservation of retrograde memory function. Cardiac arrest had no effect on anterograde memory. Exercise preconditioning provides a pragmatic measure for inducing sustainable organ protection during unpredictable cardiac events that induce prolonged global ischemic damage.

3.2. Introduction

Cardiac arrest (CA) causes whole-body ischemic injury and cell death. [7] Successful cardiopulmonary resuscitation (CPR) paradoxically confounds recovery by increasing the rate of cellular death and tissue damage through global reperfusion injury. [7] Despite decades of basic and clinical research, the prognosis after a resuscitated CA continues to be poor. [1, 4, 92] The substantial adverse effects of CA on neurologic function are a major contributor to the high incidence of morbidity and mortality following resuscitation. [7, 8, 11, 16, 22, 93, 94] A retrospective study of mortality in CA patients indicated that neurologic injury was responsible for over two thirds of the deaths.[10] Reactive oxygen species formation and activation of apoptotic and inflammatory processes during CA and resuscitation lead to tissue damage and substantial brain dysfunction. [8, 22, 93, 95, 96] Cognitive and functional impairments are prevalent in post-CA patients. Specifically, strong experimental evidence demonstrates a relationship between resuscitated CA and post-arrest impairments in memory and learning, and increases in anxiety-like behaviors. [19, 22, 80, 90, 97-99] The Cornu Ammonis 1 (CA_1) region of the hippocampus appears to be the brain region most directly responsible for these cognitive and behavioral functions and also associated with severe apoptosis following CA. [19, 21, 25]

Engaging in moderate-intensity aerobic exercise 24 to 72 hours prior to prolonged ischemic exposure can create a conditioned response that minimizes tissue damage during ischemia and reperfusion. This exercise-induced effect resembles the protection conferred by surgical induction of a series of brief, sub-lethal ischemic episodes, known as ischemic preconditioning (IPC). [39, 44] Exercise preconditioning (ExPC) prior to

transient, focal ischemia has proven to be neuroprotective. [41, 42, 100] However the value of ExPC has not been established in the severe global ischemia produced by CA.

While IPC is useful in surgical scenarios, the unpredictability of CA renders it impractical and useless as a neuroprotective defense mechanism in out-of-hospital CA. In light of this, aerobic exercise might provide the only reasonable preventative measure for inducing sustainable organ protection from the ischemic damage of CA. Accordingly, the current study investigates the value of ExPC on cognitive and behavioral factors following CA and explores its influence on neuronal preservation in the hippocampus. As with any potential therapy or preventative measure, in order to tout the medicinal value of exercise, the upper-limits of its effectiveness must be explored.

3.3. Materials and Methods

3.3.1. Subjects

Fifty-five male C57BL/6J mice 10-12 wks of age and weighing 22.79 ±0.31g were obtained from Jackson Laboratories (Bar Harbor, ME). Upon arrival the animals were randomly assigned to one of three groups: 1) the control (CON) group consisted of animals that underwent sham exercise and sham CA, (2) sham exercise plus CA (ShEx+CA), (3) exercise plus CA (Ex+CA). The animals were then subjected to behavioral testing pre- and post-CA (n=47) or sham surgery (n=8) and resuscitation. Nine animals in the CA groups did not achieve ROSC and 14 died prematurely, allowing for 24 animals (ShEx+CA, n=11; Ex+CA, n=13) to be neurologically analyzed post-arrest. After completing behavioral studies, mice were anesthetized and their brains removed for sectioning and histological analysis. All procedures were approved by the

Institutional Animal Care and Use Committee at the University of Kansas Medical Center.

3.3.2. Cardiac Arrest and Resuscitation

Mice were anesthetized with 60 mg kg^{-1} bodyweight of sodium pentobarbital delivered by intraperitoneal (i.p.) injection. The mice were orally intubated with a 20-gauge catheter, the placement of the tracheal tube was verified and mechanical ventilation was initiated (MiniVent; Hugo Sacks, March, Germany). Throughout the surgical preparation and recovery, body temperature was maintained at 37.0°C by a heating pad and monitored by a rectal thermocouple probe (Physitemp Instruments, Inc., Clifton, NJ). A 27 gauge needle was inserted into the right jugular vein for administration of potassium-chloride (KCl) and epinephrine (EPI). Arrest was induced via infusion of room temperature KCl (80 µL, 0.5 M) and discontinuation of mechanical ventilation. At 4 min 45 sec into the arrest period, the ventilator was reinitiated. At 5 min of arrest, CPR was initiated via chest compressions (approximately 300 beats per min) and administration of 5 µg of EPI in 0.25 cc saline. The jugular catheter was removed at 10 min post-recovery of spontaneous circulation (ROSC) and the wound sutured. Mechanical ventilation was discontinued after 90 min of recovery. If ROSC had not been achieved through 10 min of continuous CPR, the procedure was deemed unsuccessful and revival techniques were discontinued. A needle-probe electrocardiogram (ECG) was recorded with the use of a PC-based data acquisition system (PowerLab Inc, ADInstruments, Syndey, Australia). Animals in the CON group underwent anesthesia, intubation, mechanical ventilation, surgical preparation and insertion of the jugular catheter. These animals received injections of isotonic saline in equal volumes to the

KCl and EPI of the CA animals. The CON animals were not subjected to chest compressions.

3.3.3. Apparatus and Experimental Procedures

Mice were transported from the vivarium to the testing laboratory and allowed to rest quietly for 1h prior to the start of testing. The multi-lane treadmill, Barnes Maze and Open Field Activity (OFA) monitor were housed in the same laboratory space. When turned on, the treadmill creates a distinguishable background noise and therefore was turned on during all testing procedures regardless of whether animals were being exercise trained or not. This allowed for the standardization of auditory cues during all testing procedures. Additionally, the OFA monitor was surrounded by a white, non-transparent curtain to veil the animal from visual cues that might alter movement patterns, such as movements of the tester throughout the room, etc. Finally, the position of the Barnes Maze within the room was held constant throughout the study to maintain precise distances between the maze and external cues. All external cues were consistent throughout the study period.

3.3.3.1. Exercise Training

Exercise training was conducted using an AccuPacer 4-lane Rodent Treadmill (AccuScan Instruments, Inc., Columbus, OH). Each animal was habituated to treadmill exercise over a 5d period. Acclimation involved increasing running time by 10 min per day such that day 1 consisted of 10 min of running while day 5 consisted of 50 min at 15 m/min. Following two days of rest, the animals were exercise trained at 15 m/min for 60 min on two consecutive days, 48h and 24h prior to CA. Each treadmill session included 5 min of quiet investigation in the apparatus at 0 m/min, followed by 10 min warm up at

7 m/min, the exercise period at 15 m/min and a 5 min cool-down period at 7 m/min. The prescribed exercise intensity of 15 m/min equates to approximately 70 percent of maximum oxygen consumption and is thereby considered a moderate level of exercise. [101-103]

Sham exercised mice were placed in the treadmill at 1 m/min for the same duration as exercised mice. This treatment provided each mouse the same amount of handling, time without water or food, exposure to the stimulus of the treadmill, and potential exposure to the shock grid, without the associated stress of physical exertion.

3.3.3.2. Modified Barnes Maze

Impairments in spatial learning and memory were assessed using a modified Barnes Maze. The Barnes Maze is a circular maze designed to demonstrate deficits in developing cognitive maps using external visual cues. The Barnes Maze was originally developed for use in with rats and was thus modified in size for use with mice. Latency to escape, escape route, repeated attempts at the same hole, and number of holes investigated after identifying the escape hole were recorded. Escape was defined as all four paws in the escape box. To assess retrograde memory, each animal was tested on four consecutive days prior to CA or sham surgery, and again at 2d following surgery with two trials per testing session. Post-surgical assessment of the ability to create new cognitive maps (learning) and to retrieve those maps adequately (memory) was accomplished by changing the location of the escape hole relative to external cues. Measuring new learning occurred over four consecutive days starting at 8d post-arrest. After a latency of nine days, retention (memory) was tested at 22d post-arrest.

3.3.3.3. Open Field Activity Monitor

Emotionality and exploratory behaviors were assessed using an automated, photocell-equipped OFA monitor (AccuScan Instruments, Inc., Columbus, OH). The OFA documents rearing, resting and circling behaviors as well as total movement time, distance traveled, and margin time. Procedurally, the testing is initiated by illuminating the room, placing the animal in the center of the OFA and starting the tracking software (Fusion 3.5, Columbus, OH). The animal's movements were recorded for 10 min on three separate post-CA occasions and the data saved to a secure server to be analyzed at a later time.

3.3.4. Analysis of Neuronal Damage

At 7d post-CA, 8 mice, four per CA group, were euthanized and their brains removed and frozen at -20°C for 40 min. A 4 mm brain section beginning anteriorly at the bregma and containing the hippocampus was removed, embedded in paraffin and serially sectioned (10 µm) along the coronal axis. Apoptosis was assessed by terminal deoxytransferase-mediated dUTP nick-end labeling (TUNEL) using the *In Situ* Cell Death Detection Kit (Roche Applied Science, Indianapolis, IN). Tissue sections were incubated with TUNEL enzyme label solution and a DAPI solution for detection of nuclei and sealed with a mounting medium. TUNEL-stained tissue sections were imaged with an Olympus BX51 (Olympus America Inc, Center Valley, PA) fluorescent microscope. TUNEL-positive nuclei were counted by light microscopy under blinded conditions at 40x magnification from 5 random fields at three distinct anatomical levels (-1.28, -1.64, and -2.0 mm from bregma) and throughout the entire hippocampus (CA_1 – CA_3). The mean value of apoptotic cells was obtained from the three measurements per animal in

each of the experimental groups and expressed as a percentage of total nucleated cells.

3.3.5. Statistical Analysis

Differences between time-based measurements within individual groups were analyzed by repeated measurements of ANOVA. Differences were assessed by means of the unpaired Student *t* test between each group at every stage. Statistical analysis and graphical representation was performed with the use of SigmaStat and SigmaPlot (Jandel Corporation, San Rafael, California) with bars representing ± SEM.

3.4. Results

3.4.1. Neurologic Function

3.4.1.1. Retrograde Spatial Memory and Learning

A modified Barnes Maze was used to explore spatial working memory, spatial reference memory and spatial learning. The location of the escape box was placed with direct regard for surrounding visual cues which were held constant for the duration of the study. In analyzing working memory, the mice were scored on their ability to remember holes previously investigated and to not return to those incorrect choices (number of repeat choices). For analysis of spatial reference memory the mice were scored on their quickness to escape (latency), their ability to use visual cues to identify the location of the escape box on either the first attempt (perfect escape) or within the first two attempts (directed escape). To evaluate spatial learning this testing was conducted over four consecutive days while the location of the escape box remained in the same position on the maze relative to the external visual cues. Retrograde memory was assessed at 2d post-CA. **FIGURE 6a** shows the effects of CA and ExPC on retrograde

memory. All groups significantly decreased latency to escape after four days of testing (CON, $p<0.005$; ShEx+CA, $p<0.05$; Ex+CA, $p<0.001$). Global ischemia without preconditioning produced an increase in latency to escape at 2d that was completely negated by ExPC. No difference in latency to escape was evident at 2d between CON and Ex+CA (20.50 ±2.9 vs. 22.23 ±2.2 s, $p=0.96$), however ShEx+CA showed an increased time to escape (52.45 ±15.7 s) over both CON and Ex+CA ($p<0.05$ in both instances). There was no between-group difference in the number of directed paths, perfect trials, or repeat investigations of holes at any time point.

3.3.1.2. Anterograde Spatial Memory and Learning

The pre-arrest Barnes Maze testing was recreated in post-arrest to gauge deficiencies in creating new cognitive maps (learning) or in using and remembering visual cues (working and reference memory). In the post-arrest testing sequence, the location of the escape hole was rotated to be aligned with a different external cue, requiring new learning to take place. **FIGURE 6b** shows the effects of CA and ExPC on anterograde memory. All groups significantly decreased latency to escape by the fourth day of testing (CON, $p<0.005$; ShEx+CA, $p<0.05$; Ex+CA, $p<0.05$). No between-group differences in reference memory existed when the learning took place after CA. At 22d post-CA, latency to escape in the CON (16.67 ±3.3 s) was similar to ShEx+CA (31.25 ±10.0 s) and Ex+CA (20.50 ±3.0 s) ($p>0.05$ in all comparisons). No differences existed in the number of directed paths, perfect trials, or repeat investigations of holes at any time point.

3.3.1.3. Activity

Total distance traveled and total movement time was used to assess spontaneous locomotor activity and exploratory behavior at 8d pre-arrest to establish a baseline value, and 2d, 7d, and 22d post-CA in the OFA test. *Total Movement Time* ***(FIGURE 7a):*** At 8d pre-arrest, CON spent a significantly greater amount of time moving than ShEx+CA ($p<0.02$). The Ex+CA group was not different than CON or ShEx+CA at that time. An increase in movement was noted for Ex+CA compared to CON at 2d post-CA ($p<0.02$). No other between-group differences in movement time existed at any period. Both ShEx+CA and Ex+CA demonstrated within-group decreases in movement time between 2d and 7d ($p<0.05$ for both groups), and between 2d and 22d (ShEx+CA, $p<0.05$; Ex+CA, $p<0.001$) post-arrest. The difference between 7d and 22d was also significant for Ex+CA ($p<0.05$), but not for ShEx+CA. The CON group decreased movement time between pre-arrest and 2d ($p<0.02$), but not between any other time points.

Total Distance Traveled ***(FIGURE 7b):*** At 8d pre-arrest, CON traveled significantly farther than ShEx+CA ($p<0.02$) and Ex+CA ($p<0.05$). No between-group differences in total distance traveled existed at any post-arrest time interval. A within-group decrease in distance was noted in CON when comparing pre-arrest and 2d ($p<0.05$) and in Ex+CA when comparing 2d and 22d distances ($p<0.02$), however no further significant differences existed in any group.

3.3.1.4. Emotionality

Highly anxious mice exhibit a thigmotaxic response to a novel environment, and CA has been shown to cause high anxiety, so the OFA test was used to assess the effects of CA and ExPC on anxiety-like behaviors. The amount of time spent in the margin zone 5

cm from the wall of the cage was compared to the overall time in the apparatus. As depicted in **FIGURE 8**, an increase in margin time was noted between ShEx+CA and CON ($p<0.05$) in pre-arrest testing, but no other between-group or within-group differences were shown in margin time at or between any other time periods.

3.3.2. Neuronal Apoptosis

To associate changes in memory, learning and emotionality with hippocampal damage, and to isolate morphologic alterations attributable to ExPC, the CA_1 region of four ShEx+CA and four Ex+CA brains were subjected to TUNEL staining at 7d post-arrest and apoptosis was assessed. **FIGURE 9** demonstrates a significantly lower proportion of TUNEL-positive cells identified in the CA_1 region of Ex+CA animals compared with ShEx+CA animals (15.98 ±1.5% vs. 23.58 ±1.5%, respectively; $p<0.05$). No discernible apoptosis was present in CON.

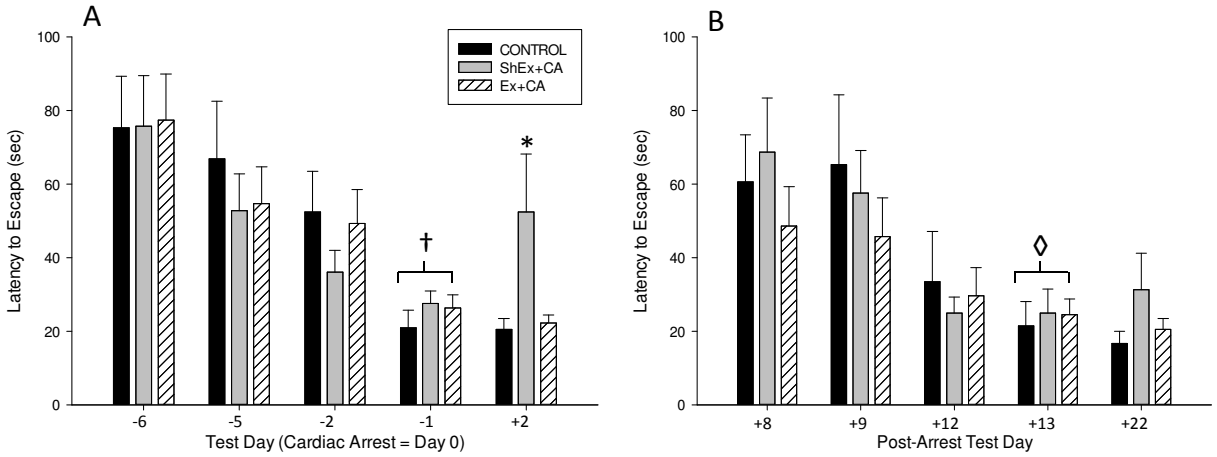
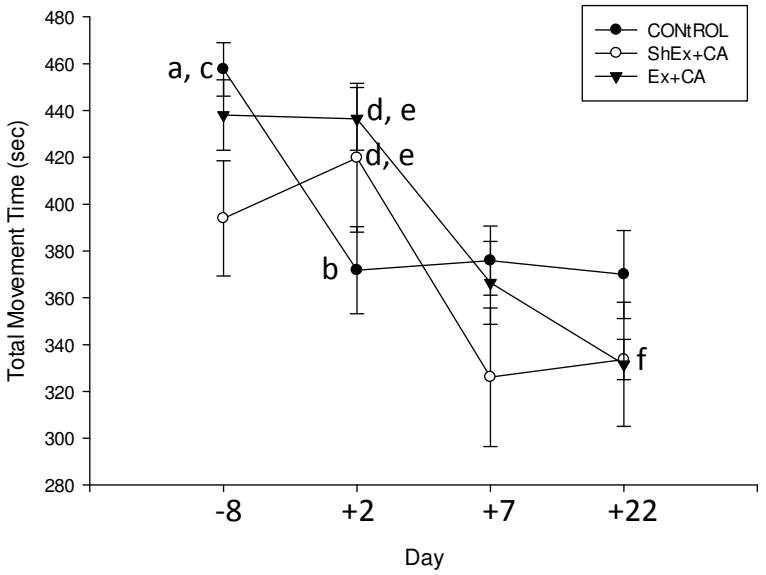


FIGURE 6. Assessment of retrograde and anterograde memory and learning was conducted via latency to escape on the Barnes Maze. All animals decreased latency to escape between -6d and -1d ($^{\dagger}p<0.02$). At 2d post-surgery, ShEx+CA animals showed an increase in latency to escape relative to CON and Ex+CA animals (A; $^{*}p<0.05$). All animals increased post-arrest latency to escape between 8d and 13d (B; $^{\lozenge}p<0.05$). No between-group differences existed in latency to escape during the post-arrest learning period (B). CON, n=8; ShEx+CA, n=11; Ex+CA, n=13.



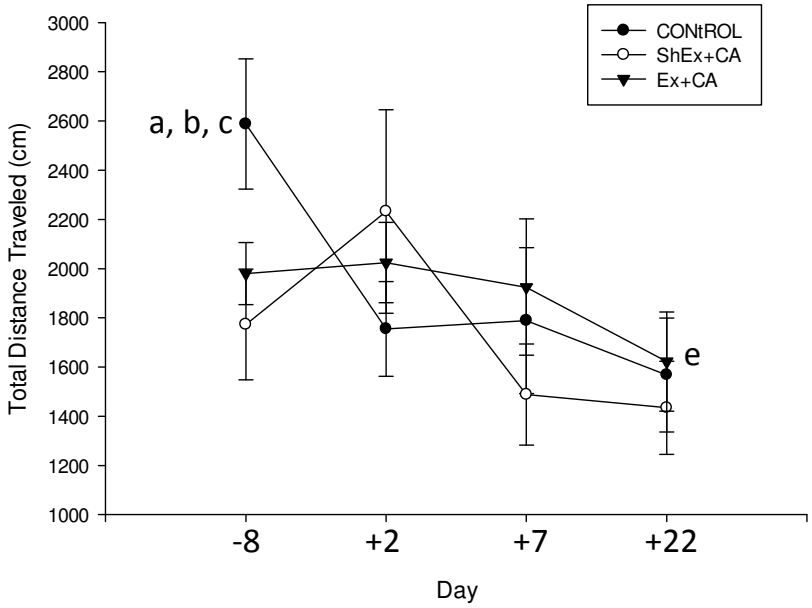


FIGURE 7. Spontaneous locomotor activity, a measure of behavior and emotionality, was gauged by assessing the total amount of time spent in motion (A), and the total distance traveled (B) in the Open Field test. Between-group differences: [a] CON vs ShEx+CA, [b]CON vs Ex+CA; and within-group differences: [c]-8d vs +48h, [d]+48h vs +7d, [e]+48h vs +22d, [f]+7d vs +22d. All values are $p<0.05$. CON, n=8; ShEx+CA, n=11; Ex+CA, n=13.

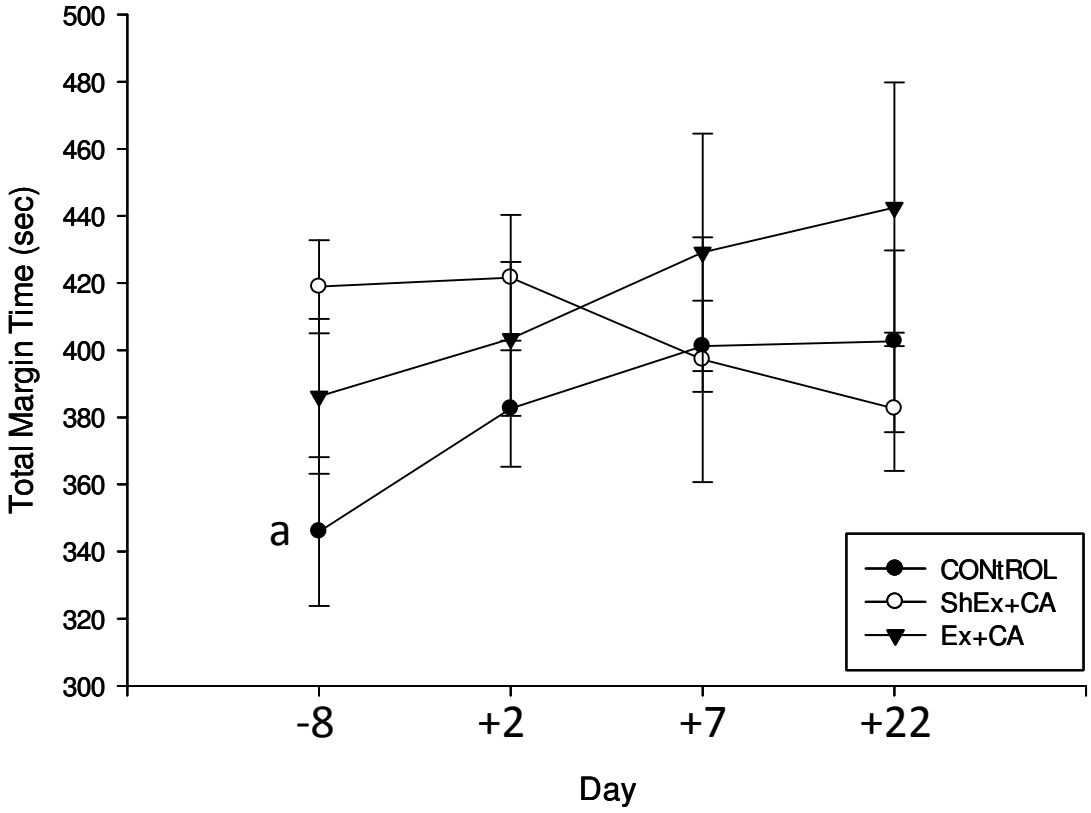


FIGURE 8. Anxiety-like behaviors were assessed by analyzing the amount of time spent within 5 cm of the walls of the testing apparatus. A between-group difference existed at -8d between ShEx+CA and CON ($^{a}p<0.02$). No group differences existed in margin time. CON, n=8; ShEx+CA, n=11; Ex+CA, n=13.

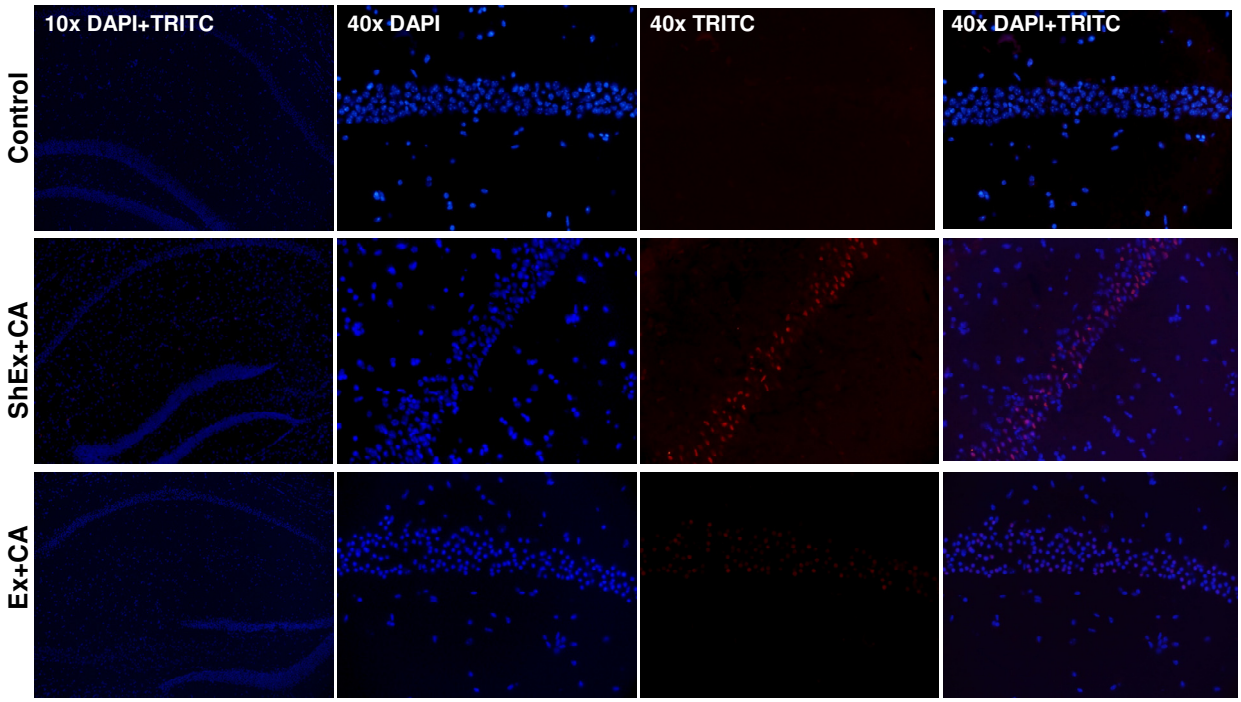


FIGURE 9. Representative images of hippocampal apoptosis determined by TUNEL staining. DAPI indicates nucleated cells; TRITC indicates apoptotic cells; DAPI+TRITC allows for identification of apoptotic cells as nucleated.

3.5. Discussion

Our results reveal a decrease in hippocampal damage with an associated sparing of brain function secondary to exercise-induced neurologic preconditioning. These findings are evidenced by the lower fraction of apoptotic cells in the CA_1 hippocampal region and supported by preserved retrograde memory in preconditioned animals compared to non-exercised mice. Our data further add to the growing body of literature showing no effect of CA on post-arrest learning and subsequent memory indicating that cognitive maps created after the arresting event are unaltered and memory recall appears unaffected by the CA.[23, 28]

The investigation of ExPC in generating a neuroprotective phenotype has largely been confined to the study of stroke and unilateral models of cerebral ischemia.[100, 104, 105] Importantly, however, these models have shown a significant improvement in post-ischemic cerebral perfusion pressure, blood flow during reperfusion, maintenance of endothelial function and a decrease in apoptosis with ExPC. Our study extends these observations to include protection during prolonged, global ischemia and reperfusion through the attenuation of hippocampal apoptosis. Furthermore, hippocampal neurogenesis has been discovered in response to voluntary exercise which could lead to enhanced spatial learning and memory.[106] Accordingly, our study demonstrated preserved cognition in exercised animals following severe ischemia.

Discrepancies exist on the duration of exercise training necessary to elicit neuroprotective benefits. Most studies using a training period of three weeks or greater have induced a protective phenotype,[40] however exercise intervals of two weeks show less conclusive evidence of protection,[61, 62] and periods of one week or less have not

demonstrated significant protection . In total, including the period of acclimation, our study generated neuroprotective changes in seven days of treadmill exercise. Thus, our study demonstrates the ability of ExPC to elicit a neuroprotective phenotype in one week of exercise training and in a global model of ischemia.

3.6 Conclusion

Future studies defining the underlying mechanisms of ExPC during CA and the duration of time that can lapse between an exercise bout and the ischemic event are crucial in generating a more complete understanding of the medicinal value of exercise. Here we have demonstrated that moderate-intensity exercise 24h prior to CA creates a conditioned, prophylactic response capable of minimizing ischemia-induced brain damage. Furthermore, exercise provides a reasonable preventative measure for inducing sustainable organ protection from unpredictable bouts of global ischemic assault.

CHAPTER 4

SUMMARY OF RESULTS AND DISCUSSION

4.1 Summary of Results

Our studies attempted to characterize survival in a murine model of potassium-induced cardiac arrest (CA) via real-time electrocardiographic (ECG) pattern recognition and assess the effectiveness of exercise in inducing a neuroprotective phenotype resistant to global ischemic injury.

1. Our results reveal definitive ECG configurations associated with survival that can be visually detected in the initial 30 s of the return of spontaneous circulation (ROSC) following potassium-induced CA. *Post-hoc* analysis substantiated these findings.

    ✓ The characterization of ECG patterns fills a void in the literature concerning the real-time assessment of the effectiveness of resuscitation efforts. Indicators of poor reperfusion, electrolyte imbalance, and severe myocardial stunning can be used to predict the likelihood of an animal developing and maintaining a sustainable rhythm. Furthermore, these indicators can be used as crucial determinants in the decision to discontinue survival efforts.

2. We demonstrated attenuated hippocampal damage with an associated sparing of retrograde spatial reference memory secondary to exercise-induced neurologic preconditioning.

    ✓ These findings extend the observations of others and expose the effectiveness of exercise preconditioning (ExPC) in combating the damage associated with prolonged ischemic exposure when the insult occurs within

24 hours of the exercise bout. These findings raise important questions regarding the duration of protection and the constraints of the mode, intensity and timing of exercise prior to a cardiac event.

4.2. Discussion

4.2.1. Clinical Significance: The double-edged sword

The clinical significance of investigating ExPC during CA has been called into question for a variety of reasons. To begin with, the likelihood of a sedentary individual coincidentally becoming physically active 24 hours prior to arrest and thus benefiting from ExPC has been doubted. To that end, it is important to consider the effects of lack of exercise on the American populace, namely the overt development of cardiovascular disease (CVD) through uncontrolled risk factors, and the strong urging of government health officials encouraging exercise.[107-109] With this knowledge and the advocacy for the medicinal value of exercise, it is plausible for a sedentary population to become suddenly active and, in fact, exacerbate a severe cardiovascular condition due to lack of knowledge on proper exercise prescription.[109, 110] An important consideration is that an increased risk of sudden cardiac death exists during and immediately following moderate to vigorous bouts of exercise regardless of an individual's exercise history.[109, 110] A study of the occurrence of cardiac arrest in a population of fitness center exercisers revealed 71 deaths over a 2 year period equating to 1 death per 82,000 members and a rate of 1 death per 2.57 million workouts.[111] The underlying significance in this study is that all the deaths occurred in apparently healthy individuals with no prior diagnosis of CVD. This risk is higher for generally sedentary individuals; it

is nevertheless existent for chronic exercisers as well. This illustrates the clinical importance of further exploration into the mechanisms and extent of ExPC.

The pathophysiology of exercise-induced cardiac events revolves around the changes in coronary blood flow during exercise and the constitution of underlying plaque formations. During exercise, coronary blood flow increases, causing a shear stress on vascular walls. This shear stress causes the endothelium to activate potent vasodilators such as nitric oxide (NO) in order to increase the lumen diameter and allow for greater blood flow. In the event of a plaque formation, the underlying endothelial dysfunction and subsequent inability to release NO inhibits the vasodilatory response. Furthermore, the structure of the plaque formation contributes considerably to the risk of an exercise-induced cardiac event. If the fibrous cap covering the plaque formation is thin, its resistance to the increase in shear stress during exercise is limited. Rupturing the fibrous cap allows the contents to spill out into the vessel leading to total vascular occlusion, and cardiac death. In a medical examination of autopsies on 141 men who died suddenly from severe coronary artery disease, 25 died during strenuous activity.[112] Of those 25 deaths, none had histological evidence of acute myocardial infarction (AMI).[112] This is suggestive of two things: 1. the plaque formation was slow in forming allowing for collateral blood flow to prevent symptomatic evidence of coronary artery disease, and 2. the fibrous plaque was too thin to withstand the shear stress applied during exertion.[112]

Cardiac arrest is not limited to sedentary individuals who suddenly become active, however. An examination of 100 men 50 to 75 years of age that had run at least 5 marathons in the previous 3 years revealed 90 percent of them to have some carotid

and peripheral artery stenosis.[113] Prior knowledge of coronary artery disease or risk factors for CVD were exclusion criteria which maintains that these apparently healthy marathon runners were harboring undiagnosed plaque developments. Should these plaques be formed with thin fibrous caps, the result could be fatal. Furthermore, a recent study of asymptomatic Los Angeles County firefighters indicated 49 percent had detectable coronary artery calcification and were significantly more likely to have plaque development in the left main coronary artery (i.e. "widow maker") than age-matched controls.[114] These examples of seemingly healthy individuals concealing occult cardiovascular disease lend further credence to the need for additional investigations of ExPC during cardiac arrest.

From our perspective, the data on the above cases are incomplete. While firefighters are known to have an elevated rate of CA compared to general populations, there is no literature discussing the rate of resuscitated CA or long-term dysfunctions following resuscitation. Likewise, it would be important to know whether a marathoner experiencing CA is more or less likely to be resuscitated and whether quality of life is impaired. Coming full circle, in light of the obesity epidemic and the rising promotion of exercise as medicine, it is important to determine if the first bout of exercise will be protective should CA occur within 24 hours.

4.2.2. Survival and Preservation: Timing is everything

Upon temporal examination of data from the exercise protocol, interesting trends in survival and apoptosis emerge. Cardiac arrest was performed on 47 mice, 9 of which did not achieve ROSC. Of the 38 remaining mice, 13 died prematurely leaving 24 mice (ShEx+CA, n=11; Ex+CA, n=13) for neurologic assessment and follow-up. No

difference in survival rates between ShEx+CA and Ex+CA existed at the study endpoint (p<0.089; **FIGURE 10**). Taking these data a step further, the largest percentage of mortality occurred at 4d, suggesting that the initial four days post-arrest are critical in determining long-term survival. More interesting, however, is the proportion of deaths per group at that time interval. Four of the 5 deaths at 4d post-CA occurred in the Ex+CA group, which sustained no further losses beyond this point. In contrast, ShEx+CA suffered losses at equal intervals throughout the testing period with the final premature death occurring at 20d post-CA. This would suggest that the critical marker for survival was *less* important in ShEx+CA than in Ex+CA. A greater conundrum is exposed when taking into consideration the significantly higher degree of apoptosis in ShEx+CA animals at 7d. It is possible, however, that this anomaly simply falls under the true-true-unrelated premise. It is factual that a large number of animals died at 4d post-arrest ($True_1$), and also that this seemingly critical marker applied more potently to the Ex+CA animals ($True_2$), but the occurrence of these two facts can plausibly be *unrelated* in the realm of physiological explanations.

In preliminary studies, a second intriguing trend in the apoptosis data relating to neurologic dysfunction emerged. While determining the optimal time to sacrifice animals for post-arrest apoptosis comparison, our data indicated minimal CA_1 apoptosis at 3h post-arrest (n=4). Furthermore, the apoptosis is confined to the most anterior sections of the hippocampus correlating with approximately -1.28 bregma (**TABLE 4**). This finding is similar to previous observations that have noted an absence of substantial neuronal death at 24h post-CA in a four-vessel occlusion model of global ischemia.[21, 115] Likewise, our 48h data are very similar to that produced via 5 min of

global ischemia.[115] Interestingly, we noted a significant *increase* in apoptosis at 48h compared to 7d ($p<0.02$) (**TABLE 5**). This is in contrast to previous findings that demonstrate the severity of neuronal degeneration progressing for at least two weeks following the ischemic event.[21, 115] While the height of neurologic functional damage in our study of cognition indicated impairments at 48h that were not persistent through the remainder of the study, this is likely attributable to differences in retrograde versus anterograde memory following cardiac arrest, and not a result of the decreased apoptosis at 7d. Therefore the conflicting data from our study and those from previously published work are unresolved.

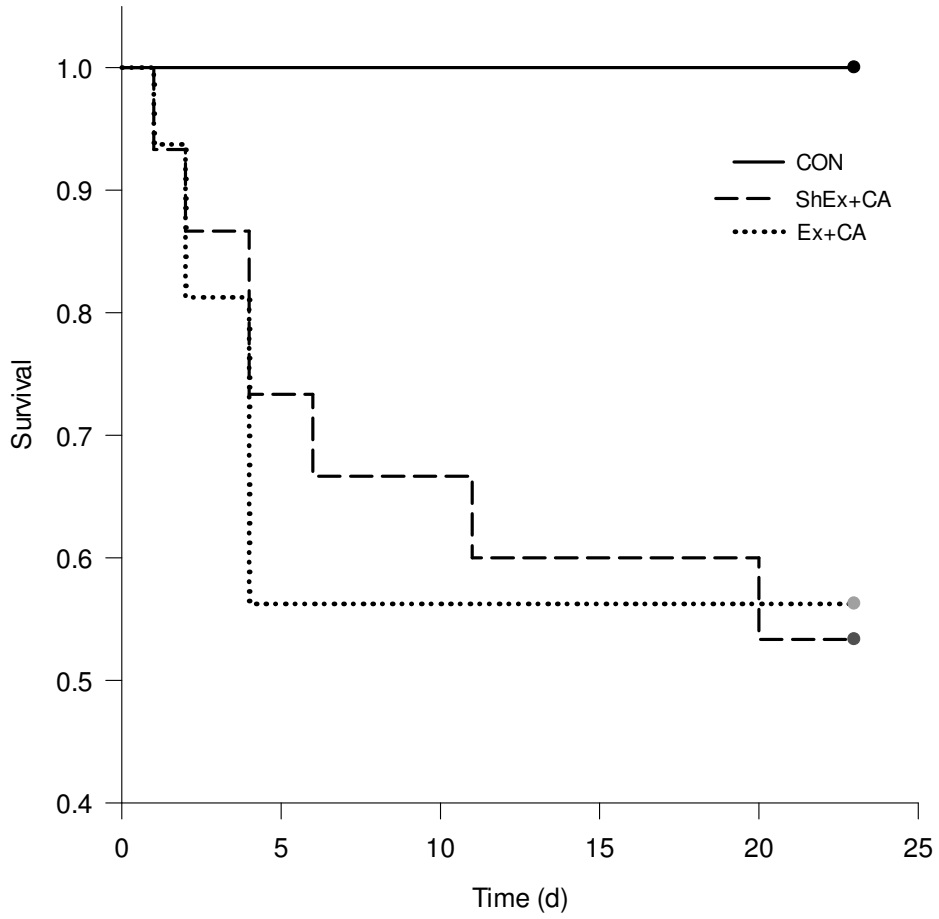


FIGURE 10. Kaplan-Meier survival curve for the duration of the post-CA recovery. Log rank analysis denied the existence of any between-group differences in survival.

TABLE 4. Analysis of apoptosis at 3h post-arrest.

	-1.28 mm bregma			-1.64 mm bregma			-2.00 mm bregma		
	Apoptotic + Nuclei	Total Nuclei	%	Apoptotic + Nuclei	Total Nuclei	%	Apoptotic + Nuclei	Total Nuclei	%
1	191	593	32.21	0	457	0.00	0	552	0.00
2	0	494	0.00	0	415	0.00	0	425	0.00
3	180	531	33.90	0	505	0.00	0	570	0.00
4	58	477	12.16	17	469	3.62	0	473	0.00
Cum	429	2095	20.48	17	1846	0.92	0	2020	0.00

		Apoptotic + Nuclei	Total Nuclei	%
Group	Total	446	5961	7.48

TABLE 5. Comparison of apoptosis assessed at 48h and 7d post-arrest. At 7d significantly fewer apoptotic cells were visible compared to 48h post-arrest ($p<0.05$).

	-1.28 mm bregma			*-1.64 mm bregma*			*-2.00 mm bregma*		
7d	Apoptotic + Nuclei	Total Nuclei	%	Apoptotic + Nuclei	Total Nuclei	%	Apoptotic + Nuclei	Total Nuclei	%
1	85	602	32.21	115	522	0.00	77	512	0.00
2	173	790	21.9	126	655	19.24	76	492	15.45
3	116	672	17.26	115	554	20.76	98	765	12.81
4	191	593	32.21	0	457	0	0	552	0
Cum	565	2657	21.26	356	2188	16.27	251	2321	10.81

		Apoptotic + Nuclei	Total Nuclei	%
Group	***Total***	***1172***	***7166***	***16.36***

	-1.28 mm bregma			*-1.64 mm bregma*			*-2.00 mm bregma*		
48h	Apoptotic + Nuclei	Total Nuclei	%	Apoptotic + Nuclei	Total Nuclei	%	Apoptotic + Nuclei	Total Nuclei	%
1	85	582	14.6	86	472	18.22	198	604	32.78
2	114	684	16.67	133	637	20.88	128	594	21.55
3	70	544	12.87	169	512	33.01	163	459	35.51
4	105	372	28.23	110	626	17.57	86	476	18.07
Cum	374	2182	17.14	498	2247	22.16	575	2133	26.96

		Apoptotic + Nuclei	Total Nuclei	%
Group	***Total***	***1447***	***6562***	***22.05***

4.2.3. Electrocardiographic Interpretation: How wide and long and high and deep

An exhaustive literature search was unable to uncover quantitative data regarding the effects of hypoxia or hyperkalemia on murine ECG variables. One study of focal ischemia-reperfusion which included limited quantitative data was found.[77] This study did, however, include substantial ECG images that were used to correlate our findings of pattern recognition.[77] A striking similarity exists in waveform appearance between the post-reperfusion image associated with 24h of ischemia and the initial waveform at ROSC of survivors in our study (**FIGURE 11**).[77] Though not quantified, Preda and Burlacu[77] note the significant changes of T wave inversion, pathologic Q waves and microvoltage abnormalities. Interestingly, the survivors of our study progressed out of the wide QRS complex pattern and into a healthy, biphasic waveform whereas the animals in the reference study maintained this ECG alteration.[77]

A mnemonic device that relies on four essential questions was used to aid in ECG pattern recognition: how wide, how long, how high and how deep? (Reference **FIGURE 4** and **FIGURE 5** for illustrations of each wave pattern described below).

- ✓ *How <u>wide</u> is the QRS complex*: As shown previously, a wide QRS complex is characteristic of non-survivors. While survivors tend to display a wide QRS complex at the onset of ROSC it rapidly narrows giving way to a shorter RR interval and faster heart rate (HR). Preda and Burlacu[77] recognized this wide QRS complex to be associated with poor reperfusion following prolonged ischemia. In our study, the initial wide QRS of the survivors likely represents a period of re-establishing perfusion throughout the myocardium. Furthermore, the

persistent wide QRS complex of non-survivors likely reflects an idioventricular rhythm.

- ✓ *How <u>long</u> is the RR interval:* Not only is the length of the RR interval key in determining survivorship, but so too is the beat-to-beat regularity of the RR interval. Irregularity and prolongation are key factors for non-survivors and generally predict the development of arrhythmias leading to complete heart block and asystole.
- ✓ *How <u>high</u> is the ST-Segment (J-point)*: The hallmark of acute myocardial infarction (AMI), ST-segment changes indicate myocardial contractile disruptions from either ischemic injury or myocardial stunning. Interestingly, non-survivors typically lack a distinct ST-segment or T-wave altogether. In contrast, the ST-segment of survivors generally cycles from elevation to depression in the immediate recovery phase and back to elevation by 15 min post-CA. Amplitude could also be considered in the *"how high"* category as survivors typically present with low amplitude, biphasic waves and non-survivors with high amplitude monophasic waves.
- ✓ *How <u>deep</u> is the Q wave:* The characteristic QS wave seen in non-survivors is distinctive in its depth, typically ranging between -0.75 and -1.0 mV

No difference exists in quantitative measures of ECG waveforms and patterns between Ex+CA and ShEx+CA (**TABLE 6**). Because of the similarity in ECG quantification at ROSC, the Ex+CA and ShEx+CA groups were separated based on survival and non-survival. However, between- and within-group differences in HR are, in fact, evident at the onset of ROSC which persist to the study endpoint of 23d post-CA.

In the initial 30 min post-ROSC, both Ex+CA and ShEx+CA experience an increase in HR over baseline. This is to be expected as one of the primary identifying factors for survival is a shortened RR interval and corresponding increased HR compared with non-survivors. Interestingly, at 5 min post-CA, ShEx+CA have a significantly *lower* HR than Ex+CA ($p<0.05$). This difference normalizes by 10 min post-arrest and HR levels are comparable through the remainder of the early recovery phase **(TABLE 7**).

Extending the HR analysis further, our data show a significant within-group increase at 2d, 7d and 22d post-CA compared to baseline (pre-arrest) in Ex+CA ($p<0.001$, 0.05, and 0.05, respectively). In contrast, an increase in HR over baseline is apparent in ShEx+CA only at 7d post-arrest ($p<0.05$). Inexplicably, CON also experienced a significant increase in HR over baseline at all time points following sham surgery (2d and 22d, $p<0.05$; 7d, $p<0.01$) (**TABLE 8**). These HR were obtained prior to echocardiographic assessment of cardiac function, immediately following sedation with 3.0 percent isoflurane. A study of the hemodynamic effects of isoflurane in C57Bl/6J mice demonstrates an increase in HR in response to sedation that persists for 120 min.[116] Unfortunately, all echocardiograms in our lab are conducted following the isoflurane procedure and would not account for the lower HR at baseline or the inconsistency in HR found in the ShEx+CA animals (echocardiographic data not shown due to large variability). Differences did exist, however in the time of day the echocardiograms were conducted and in the testing procedures the mice were exposed to prior to echocardiographic assessment. Thus strong circadian rhythm influences could be responsible for the increases in HR, or an increase in sympathetic drive as a

result of handling and exposure to stimuli outside the normal cage activity might explain the elevated HR.

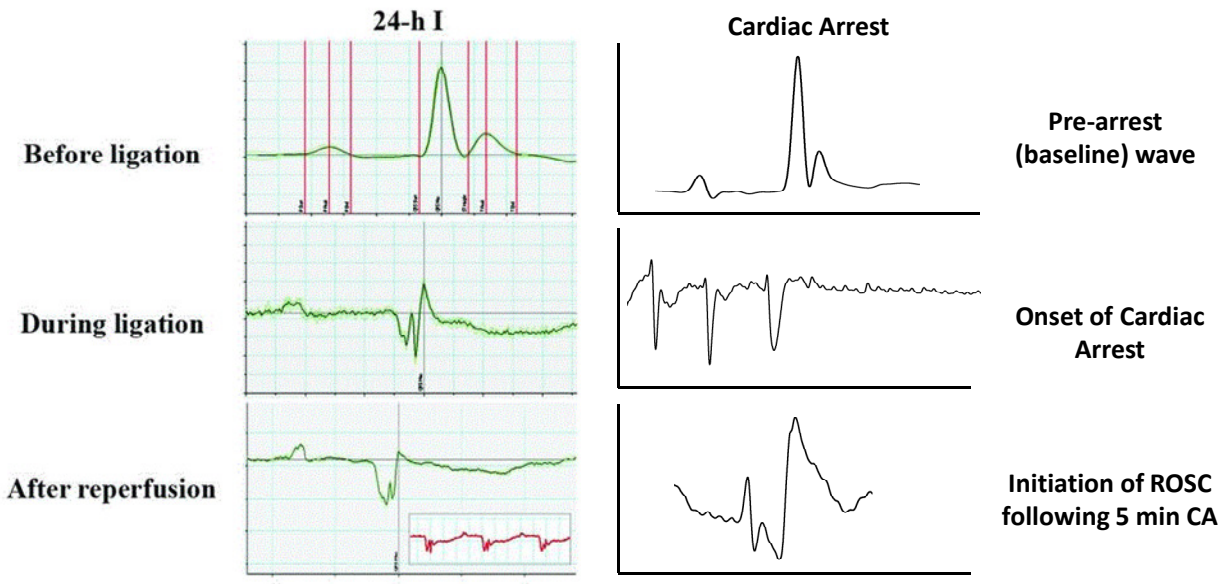


FIGURE 11. Comparison of ECG characteristics between a 24h coronary ligation and 5 min cardiac arrest (left and right panels, repectively). Left panel modified and reprinted with permission, Preda and Burlacu.[77]

TABLE 6. A comparison of quantitative measures of ECG waveforms between ShEx+CA and Ex+CA at the onset of ROSC and through the initial 90 s. No between-group differences existed. ShEx+CA, n=6; Ex+CA, n=11.

	Baseline		Onset of ROSC (T1)		30 s after ROSC (T2)		60 s after ROSC (T3)	
	ShEx+CA	Ex+CA	ShEx+CA	Ex+CA	ShEx+CA	Ex+CA	ShEx+CA	Ex+CA
RR (ms)	170.47 (16.34)	155.07 (5.70)	148.67 (3.53)	152.52 (5.81)	195.14 (23.06)	110.15 (3.31)	108.12 (2.26)	114.07 (2.36)
HR (bpm)	370.82 (38.04)	394.85 (17.78)	432.83 (12.65)	424.42 (10.64)	346.18 (31.63)	548.57 (15.28)	556.17 (11.29)	528.41 (11.17)
PR (ms)	33.46 (0.76)	34.26 (0.69)	21.24 (0.80)	24.23 (1.58)	24.36 (3.22)	36.60 (1.72)	35.78 (0.97)	38.21 (1.32)
QRS (ms)	11.27 (0.64)	11.11 (0.23)	22.88 (1.68)	24.07 (1.90)	24.34 (2.43)	14.19 (1.35)	11.92 (0.49)	14.80 (1.45)
QT (ms)	21.48 (2.41)	18.98 (0.69)	43.66 (3.16)	41.16 (2.36)	42.08 (4.03)	23.32 (2.44)	25.40 (0.00)	28.12 (3.11)
QTc (ms)	54.40 (8.61)	48.47 (1.87)	123.01 (12.29)	108.40 (7.93)	92.78 (8.95)	68.05 (7.80)	75.06 (0.00)	83.88 (8.59)
Q_{Amp} (mV)	5.53 (1.84)	9.05 (2.83)	-14.98 (58.97)	-113.18 (38.02)	-235.36 (115.08)	10.34 (26.24)	44.08 (26.95)	35.16 (19.27)
ST (mV)	38.82 (9.74)	48.17 (9.95)	31.12 (41.99)	5.49 (25.02)	21.21 (62.56)	-5.48 (24.19)	21.94 (17.27)	84.17 (20.64)

TABLE 7. Heart rate response between ShEx+CA and Ex+CA through the first 30 min of recovery. [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.005$; [d] $p<0.001$ vs BSL; [e]$p<0.05$ vs Ex+CA. BSL, Baseline.

		Post-Arrest			
	BSL	5min	10min	15min	30min
ShEx+CA	392.00	495.27[b,e]	512.10[d]	542.18[c]	501.40[a]
SEM	±17.96	±16.65	±15.85	±27.10	±40.62
Ex+CA	374.86	545.00[d]	487.00[c]	517.71[d]	522.83[d]
SEM	±12.86	±11.56	±19.61	±10.53	±26.70

TABLE 8. Heart rate response throughout the 23d survival period. [a] $p<0.05$; [b] $p<0.01$; [c] $p<0.005$; [d] $p<0.001$ vs BSL. BSL, Baseline.

	BSL	+2d	+7d	+23d
Heart Rate (bpm)				
Control	432.00 ± 17.68	490.50 ± 11.70[a]	515.13 ± 10.99[b]	490.38 ± 17.95[a]
ShEx+CA	450.63 ± 19.83	477.46 ± 17.66	502.90 ± 12.28[a]	494.14 ± 17.45
Ex+CA	416.91 ± 15.70	503.95 ± 17.00[d]	489.29 ± 21.12[a]	475.87 ± 18.27[a]

4.2.4. Modeling Cardiac Arrest: Choosing battles wisely

Four major models of CA have been developed for experimental investigations: exsanguination, electrical induction of ventricular fibrillation (VF), asphyxiation, and KCl.

Exsanguination: Procedurally, exsanguination creates the greatest surgical challenge due to the fine line between generating a model of hypovolemic shock and full cardiac arrest that is wholly dependent on the amount of blood extracted and the duration of extraction.

Ventricular Fibrillation: The murine model of VF presents complications with the rate of myocardial excitation. The normal murine HR ranges between 450 and 600 beats per minute, on average, thus electrical stimulation would have to induce a rate *higher* than that achieved through normal excitation. More importantly, VF induced by electrical stimulation differs from that instigated by coronary occlusion (i.e. a clinical model) in rates of resuscitation, time to ROSC, and post-resuscitation myocardial dysfunction.[117]

Asphyxiation: Asphyxial arrest is rarely observed in an adult population, thus the use of this experimental model is limited in generalizability to a pediatric population or those with severe pulmonary complications. Furthermore, brain damage tends to be more severe in asphyxial CA than in the other models, perhaps due to a slow progression from hypoxemia to anoxia before complete circulatory arrest.[7, 118]

KCl: Finally, the use of KCl as an arresting agent presents with the potential for residual KCl to remain in the system and augment damage during CPR and reperfusion. Lingering KCl could alter biochemical processes related to electromechanical recovery of cardiac function and prolong neurologic damage even after ROSC has been

achieved.[80] However, KCl does provide immediate and persistent asystole which allows for a more accurate analysis of total arrest time. The ability to achieve CA for a precise period prior to the start of CPR allows for consistency between animals.

Clearly, all models of CA have limiting factors, not the least of which is the ability to generalize to a clinical population. Rarely are these models preceded by development of AMI which, clinically, accounts for the majority of CA cases.[2] Furthermore, the time of day in which arrests are induced is a confounding factor in data acquisition. Research has indicated a significant increase in hippocampal damage when the arrest occurs 6 to 8 h into the light phase of the light:dark cycle compared to arrests induced in the dark phase.[119] In a surgical scenario in which all mice are arrested in a single day without account for circadian cycling, some mice will inevitably incur greater damage simply due to the timing of surgery rather than due to the physiologic insult. Interestingly, the temporal factor has clinical relevance and therefore deserves heightened attention and consideration in experimental models.[120]

Beyond choosing the methodology that most appropriately serves the needs of the study, the animal strain plays a significant role in the success of the study. In our model of CA we used C57Bl/6J mice for two basic reasons: First, the C57Bl/6J mouse is extensively used as the background strain for the generation of transgenic and gene ablation models. This allows for a higher degree of comparability when examining molecular pathophysiology and microadaptations. Secondly, the development of a CA model with prior induction of AMI is possible, though technically challenging, in this particular strain as it is susceptible to diet-induced obesity, type 2 diabetes and atherosclerosis, all of which are predisposing factors in cardiovascular disease

development.[121-123] Unfortunately, the premise of the current study revolved around an exercising model of preconditioning and C57Bl/6J mice are categorically poor performers in forced exercise.[103, 124, 125] A study of differences in response to forced treadmill exercise of 13 commonly used mouse strains demonstrated C57Bl/6J mice to incur the highest number of contacts with the shock grid located at the end of the treadmill belt, and the slowest maximal run speed.[103, 125] In this battle, the ability to cross-reference the neurologic results and physiologic responses to global ischemia with previous studies in our lab held greater influence than exercise performance in determining the mouse strain used.

CHAPTER 5

FUTURE STUDIES

5.1. From Exercise to Arrest: Refining the Model

Future studies following this project should consider the multitude of methodological variations that can enhance the translatability of this model to a clinical setting and ensure clinical relevance. The copious amounts of data that can be obtained during these studies should also not be overlooked. In titling this project Organ Protection rather than Neurologic Protection we acknowledge the whole-body effects of cardiac arrest. To that end, tissue samples from the kidneys, liver, lung and skeletal muscles of all animals have been harvested and preserved in a -80℃ freezer for future analysis. These tissue samples provide an ideal starting point for complementary studies in this line of research. **FIGURE 12** outlines several methodological variables worthy of consideration in future studies which should also take into consideration the global effects of cardiac arrest.

5.1.1. Inducing Arrest through Myocardial Infarction

With the majority of arrests occurring through cardiac etiology it seems intuitive that a more clinically relevant model of arrest would use cardiac pathology to induce arrest. While this is challenging in a small animal model of arrest, utilizing animals prone to the development of cardiovascular disease (CVD) might alleviate the difficulty of coronary ligation combined with arrest induction. The pathophysiology of CVD and CA are intensely complicated such that the removal of one stressor could alter the mechanistic response entirely. In studying arrest in the absence of underlying cardiac etiology, it is possible for researchers to be chasing clinically irrelevant processes in pathogenesis.

5.1.2. Diurnal adjustments

As stated previously, experimental evidence showed a strong time-of-day variation in hippocampal ischemic damage.[119] In order to conduct the arrests at different points in the light:dark cycle without disrupting the normal working patterns of the surgeons, these researchers altered the light exposure time of the animals via use of a light-tight housing cabinet.[119] The significantly elevated ischemic damage observed in animals who underwent CA during the light phase of the light:dark cycle is in direct accordance with clinical findings.[119, 120] Martinez found survival-to-hospital discharge varies according to the time of day and the day of the week that the arrest occurs.[120] In particular, a significantly higher incidence of CA occurs between 8am and 10am. Likewise, there is significant variation across the days of the week such that more arrests occur on Saturdays.[120] Interestingly, with regard to survival-to-discharge, the lowest rates of survival occur in the overnight hours of midnight to 6am.[120] This body of evidence substantiates the need to keep the point in the light: dark cycle consistent in future studies to reduce confounding variables in data analysis.

5.1.3. Latency from Exercise Preconditioning to Arrest

Lennon et al[58] noted exercise preconditioning (ExPC) remaining cardioprotective for nine days whereas IPC affords a four day window of protection.[38] Future studies should extend the results of ischemia/reperfusion studies to examine the effects of ExPC during CA. Furthermore, no studies have examined the protracted effects of ExPC on neuroprotection which presents a new area of focus for research in ExPC and CA.

5.1.3. Age

Interestingly, a majority of the investigations on cardio- and neuroprotective measures and mechanistic underpinnings use mice or rats that would fall into the young adult age range. Clinically, this is *not* the population at greatest risk for CA or manifestations of CVD. It would be exceedingly useful in creating a more generalizable model if an older animal were used in the study of CA. Importantly, C57Bl/6J mice create ideal models of increased CVD risk. Allowed chow *ad libitum* they tend to overeat and are prone to weight issues in later stages of life. Additionally, when fed a high fat diet they are increasingly more susceptible to obesity, type 2 diabetes and atherosclerosis. Using this model, rather than a healthy young adult, would significantly increase the clinical relevance. Special precautions would need to be in place for the use of an intensive exercise program in a population reflective of CVD.

5.1.4. Gender

Substantial evidence supports the cardio- and neuroprotective benefits of estrogen in the face of ischemic insult.[126-129] This leaves the premenopausal population relatively underinvestigated in the realm of preconditioning measures. The potential for capitalizing on the benefits of exercise and investigating the potential for a synergistic effect between exercise and estrogen is largely untapped. Additionally, as stated previously, most studies of preconditioning look only as far as transient, focal ischemia. It is plausible that a prolonged, severe ischemic event such as CA would outstrip the protection laid down by estrogen. In this scenario, ExPC might be able to assist in maintaining organ function.

5.2. Conclusion

Joan Welsh said, “A man’s health can be judged by that which he takes two at a time – pills or stairs.” The mounting evidence in support of the medicinal value of exercise is hard to ignore, yet the prescription of exercise for disease treatment and prevention is inexplicably low. Here we have shown the power of exercise in conferring protection during severe, global ischemia. This report substantiates the growing body of research that promotes exercise as the only practical, preventative measure for inducing sustainable organ protection during unforeseen cardiac events.

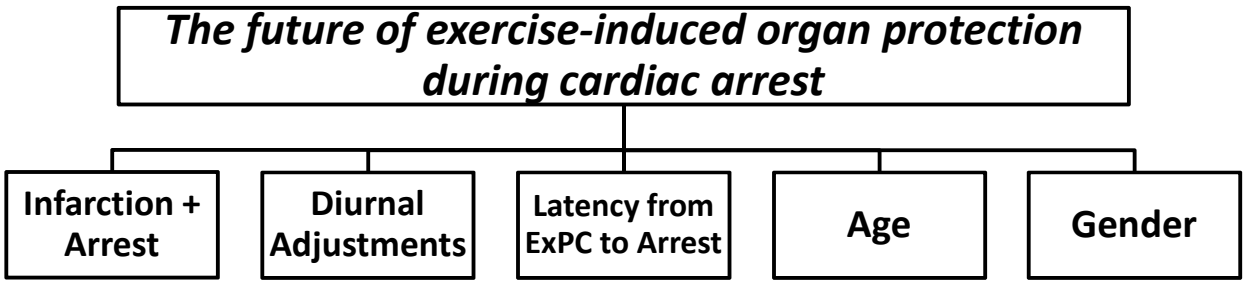


FIGURE 12. Variables for consideration for future studies of exercise preconditioning during cardiac arrest.

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